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Clinical consequences of pancreatic exocrine insufficiency

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”A new idea must not be judged by its immediate results”

Nikola Tesla

Clinical consequences of pancreatic exocrine insufficiency

THESIS FOR DOCTORAL DEGREE (Ph.D.)

Tuesday the 22th of June, 2021, 09.00 am

By

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To Mikael

ABSTRACT

Background: Chronic pancreatitis (CP) is characterized by a chronic inflammation with fibrosis and irreversible morphological changes that can result in permanent structural changes with a loss of exocrine and endocrine pancreas function. Exocrine dysfunction leads to reduced production of pancreatic digestive enzymes which is the reason of maldigestion and malabsorption of ingested nutrients like fats, microelements, vitamins and proteins which can lead to osteopathy.

Aims: To determine the prevalence of fat-soluble vitamin deficiency in CP patients by a meta-analysis. To evaluate the prevalence of osteopenia and osteoporosis in patients with CP and investigate the correlation between bone mineral density (BMD) and CP features, and vitamin D and pancreatic exocrine insufficiency (PEI). To evaluate the prevalence of PEI in patients with Crohn`s disease (CD). To evaluate the prevalence of PEI and gastrointestinal symptoms in patients with Sjögren´s syndrome (SS).

Methods: In ***paper I*** we performed a systematic review and meta-analysis. MEDLINE was searched up to January 2016 for case series and case-control studies reporting of fat-soluble vitamin deficiency in CP patients. In ***paper II***, a multicenter cross-sectional study was performed in CP patients. Key clinical and biochemical variables were recorded. PEI was assessed by fecal elastase (FE-1) and standardized osteodensitometry was performed by DEXA. In ***paper III***, patients with CD were recruited at Karolinska University Hospital and demographic, clinical and laboratory data were analyzed. PEI was assessed by FE-1. In ***paper IV***, a cross-sectional monocenter study including 57 patients with well-characterized primary SS. Patients were recruited from the Department for Rheumatology at Karolinska University Hospital in Stockholm, Sweden, between June and December 2019. Key clinical characteristics were recorded. Pancreatic exocrine insufficiency (PEI) was assessed by fecal elastase FE-1 and ^{13}C-mixed triglyceride breath test (^{13}C-MTG-BT). PEI was defined as FE1 <200 μg/g and a cumulative ^{13}C-exhalation <20.9%, respectively. The presence and severity of gastrointestinal symptoms were assessed by a well-established and validated survey on the basis of seven-point Likert scale (SSRS/GSRS-IBS). Results of the questionnaire were compared with sex and age-matched controls.

Results: In ***paper I***, twelve studies with 548 patients were included. With a random-effect model, the pooled prevalence rate of vitamin A, D and E deficiency were 16.8%, 57.6% and 29.2% respectively, with considerable heterogeneity (I2 . 75%, 87.1% and 92%). Only one study evaluated vitamin K deficiency. The pooled OR for vitamin D deficiency in CP cases compared with controls was 1.17 (95% CI 0.77-1.78). Sensitivity analyses showed lower prevalence of vitamin A and E, and higher prevalence of vitamin D deficiency in high-quality studies. The rate of pancreatic exocrine insufficiency did not seem affect the deficiency rates, while the use of different cut-offs influences results and heterogeneity for vitamin E, but not A. In ***paper II***, 188 consecutive CP patients were enrolled at 6 centres (67% M; mean age 60 years). Osteopenia was diagnosed in 42% and osteoporosis in 22% of cases. The underlying etiology was alcohol in 43% of cases, and 18% had severe CP. Fifty-three % of patients had PEI. The mean value of vitamin D was 21 ng/ml and 56% of cases had vitamin D insufficiency. There was no correlation between vitamin D levels and fecal elastase-1 levels or the t-score (spine or femur). Alcoholic etiology was associated with higher risk of having low levels of fecal elastase-1 ($p=0.02$) and with lower level of vitamin D ($p=0.001$) but not with osteopenia or osteoporosis. BMI was lower in patients with osteoporosis ($p=0.001$). In ***paper III***, 20 patients were included comprising 13 (65%) males and 7 (35%) females with a mean age of 48.3+-1.4 years. The mean duration of CD was 15.7+- years (range 1-40 years). There were 11 (55%) patients without history of bowel surgery and 9 (45%) patients after ileocecal resection. FE-1 test was normal in all patients. In ***paper IV***, fifty-seven patients with primary SS were included in the study, comprising 92% females with a median age of 63 years. In total, (87%) 50/57 of SS patients were tested for FE-1 and all had normal results. All patients who underwent a ^{13}C-MTG-BT (21/57; 37%) had a normal cumulative ^{13}C-exhalation (>20.9%). Compared to the control group, significantly more patients suffered from GI symptoms ($p<0.01$ in all 11 items). The same number of patients noted moderate to severe loose bowel movements (38%) and constipation (38%). Eleven GI symptom parameters were compared to controls, the highest odd ratios were noted for the following moderate to severe symptoms: bloating (OR: 27.9, 95% CI: 9.81-91.9), feeling of incomplete emptied bowel after defecation (OR 21.4, 95%: 6.95-75.8), and abdominal pain relieved by bowel action (OR 17.8, 95% CI: 6.04-62.2).

Conclusions: In our meta-analysis, fat-soluble vitamins deficiency is frequent in CP patients, but there is considerable heterogeneity between different studies. With regards to vitamin D, there are few case-control studies and no apparent increased deficiency risk in CP vs controls.

The present data in paper II confirm a high rate of osteopathy in CP patients. There was apparently no correlation between BMD, pancreatic exocrine function, severity of chronic pancreatitis or vitamin D levels. In paper III, FE-1 was normal in all patients with CD which strongly indicated absence of PEI in this group of patients. In paper IV, according to our study, the vast majority of SS patients suffered from IBS-like GI symptoms that cannot be attributed to pancreatic exocrine insufficiency.

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LIST OF SCIENTIFIC PAPERS

I. **Deficiency of fat-soluble vitamins in chronic pancreatitis: a systematic review and meta-analysis**
Emma Martínez-Moneo, Serena Stigliano, Aleksandra Hedström, Aleksandra Kaczka, Marko Malvik, Alexander Waldthaler, Patrick Maisonneuve, Peter Simon, Gabriele Capurso.
Pancreatology, 2016; 16(6): 988-994

II. **Vitamins D and K as factors associated with osteopathy in chronic pancreatitis: A prospective multicentre study (P-BONE study)**
Serena Stigliano, Alexander Waldhaler, Emma Martinez, Luana Lionetto, Stuart Robinson, Marko Malvik, Aleksandra Hedström, Aleksandra Kaczka, Marius Scholdei, Stephan Haas, Maurizio Simmaco, Gianfranco Delle Fave, Matthias Löhr, Peter Simon, Gabriele Capurso.
Clin Transl Gastroenterol. 2018; 9(10): 197

III. **Pancreatic exocrine insufficiency in patients and Crohn`s disease**
Aleksandra Hedström, Corinna Steiner, Roberto Valente, Stephan L. Haas, Matthias Löhr, Miroslav Vujasinovic
Minerva Gastroenterol Dietol. 2020; 66(1): 17-22

IV. **High prevalence of gastrointestinal symptoms in patients with primary Sjögren´s syndrome cannot be attributed to pancreatic exocrine insufficiency**
Aleksandra Hedström, Marika Kvarnström, Greger Lindberg, Sandra Alsabeah, Hanna Alsabeah, Nelson Ndegwa, J.-Matthias Löhr, Stephan L. Haas*, Miroslav Vujasinovic* (*equally contributed)
Manuscript

OTHER PUBLICATIONS

Conservative Treatment of chronic pancreatitis

Stephan L. Haas, Fredrik Lindgren, Lars Enochsson, **Aleksandra Hedström**, Fredrik Swahn, Ralf Segersvärd, Urban Arnelo

Dig Dis. 2013; 31(1): 43-50

Autoimmune pancreatitis-new kid on the block

J.-Mathias Löhr, Urban Arnelo, Marco Del Chiaro, Stephan L. Haas, **Aleksandra Hedström**, Fredrik Lindgren, Nikolaos Kartalis, Ralf Segersvärd, Caroline Verbeke

Gastrokuriren nr. 3, 2015

"Exokrin pankreasinsufficiens som differentialdiagnos vid diarré"

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Zinc deficiency in patients with chronic pancreatitis

Miroslav Vujasinovic , **Aleksandra Hedström** , Patrick Maisonneuve, Roberto Valente , Henrik von Horn H, J.-Matthias Löhr, Stephan L. Haas.

World J Gastroenterol. 2019; 25(5): 600-607

LIST OF ABBEREVIATIONS

AIP	Autoimmune pancreatitis
AP	Acute pancreatitis
BMD	Bone mineral density
BMI	Body mass index
CD	Crohn´s disease
CP	Chronic pancreatitis
CT	Computed tomography
DXA	Dual-energy X-ray apsorptiometry
EUS	endoscopic ultrasound
ERCP	Endoscopic retrograde cholangio-pancreatography
GSRS	Gastrointestinal Symptom Rating Scale
IBD	Inflammatory bowel disease
ICD	International Statistical Classification of Diseases and Related Health Problems
MRCP	Magnetic resonance cholangiopancreatography
MRI	Magnetic resonance imaging
PEI	Pancreatic exocrine insuficiency
PSC	Primary sclerosing cholangitis
SS	Sjögren´s syndrome
SSA	anti–Sjögren's-syndrome-related antigen A autoantibodies

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1. INTRODUCTION

1.1 Pancreas anatomy and physiology

The pancreas is a digestive gland that is located deep in the retroperitoneum crossing the first and second lumbar vertebra of the spine. The pancreas lies between the duodenal C on the right and the splenic hilum on the left. Parts of the pancreas termed head, neck, body and the tail (***Figure 1***). The largest part of the pancreas is represented by the pancreatic head. (1) The volume of the adult pancreas is approximately 70-80 cm^3 and decreases during the ageing process.(2)

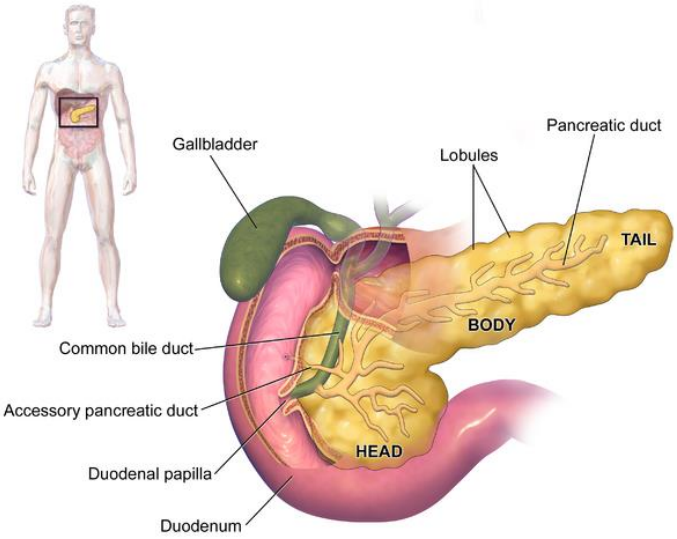


Figure 1. ***Anatomy of the pancreas.*** *Blausen.com staff (2014). "Medical gallery of Blausen Medical 2014". WikiJournal of Medicine 1 (2).Creative Commons CC BY.*

The pancreas can be divided in the exocrine and endocrine pancreas. The exocrine pancreas produces enzymes that are secreted into the gastrointestinal tract, whereas the endocrine pancreas synthesizes hormones which have direct access to the blood circulation.
The exocrine pancreas is represented by acinar cells which are forming a functional unit called acinus (***Figure 2***). Acinar cells are highly specialized polarized cells with a high capacity for protein synthesis and produce and actively secrete all pancreatic enzymes that are required for digestion (***Table 1***). The majority of enzymes are synthesized as inactive pro-enzymes which reach the duodenum by the pancreatic duct system.

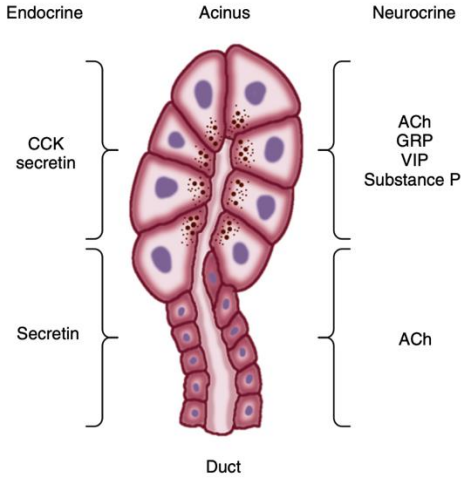


Figure 2*. Secretin and cholecystokinin (CCK) are the two main hormones regulating pancreatic secretion. Secretin strongly stimulates the production of bicarbonate rich pancreatic juices by its effect on the ductular cells of the pancreas but has even a stimulatory effect on acinar cells. CCK stimulates production and secretion of pancreatic enzymes. Parasympathetic vagal innervation of the pancreas stimulates both enzyme and bicarbonate production.*
From: Sleisenger and Fordtran's Gastrointestinal and Liver Disease 11th edition 2020. With permission from Elsevier.

PROENZYMES*
Anionic trypsinogen
Cationic trypsinogen
Chymotrypsinogen (A, B)
Kallireinogen
Mesotrypsinogen
Procarboxypeptidase A (1, 2)
Procarboxypeptidase B (1, 2)
Proelastase
Prophospholipase A_2
ENZYMES
Amylase
Carboxylesterase
DNase
Lipase (TG lipase)
RNase
Sterol esterase

Table 1*: A large range of different pancreatic enzymes are produced and secreted by acinar cells. Inactive proenzymes are activated in the duodenum. All enzymes are stored in intracellular zymogen granules to prevent activation in the pancreas.*
From: Sleisenger and Fordtran's Gastrointestinal and Liver Disease 11th edition 2020. With permission from Elsevier.

Several safety systems have emerged during evolution to prevent activation of pancreatic proenzymes in the pancreas which would trigger autodigestion resulting in pancreatitis. Inactive proenzymes become activated after reaching the duodenum by enterokinase which is produced in the duodenum. Enterokinase-mediated activation of trypsinogen plays a key role in the activation process ***(Figure 3***).

Active trypsin is able to activate several different proenzymes. By contrast, other enzymes of the exocrine pancreas are produced in their active forms (amylase, carboxylesterase, DNAse, RNAse). The pancreas is the only source for lipase, whereas other enzymes are produced even in other organs. For example, salivary glands secrete significant amounts of amylase in order to metabolize starch and glycogen.

Synthesis and secretion of pancreatic enzymes is regulated by the neurohumoral system. Innervation of the pancreas by vagal nerves stimulates - via acetylcholine (ACh) – the secretion of pancreatic enzymes following increase of intracellular calcium (Ca^{2+}).

Cholecystokinin (CCK) is the main hormone that stimulates pancreatic enzyme secretion via binding to receptors of the acinar cells. A meal passing the stomach and reaching the duodenum stimulates CCK release in the duodenum and subsequent pancreatic secretion (***Figure 4***). Other hormones such as Gastrin-releasing peptide (GRP), vasoactive intestinal peptide (VIP) and substance P have additional stimulatory function but play a less important role in the regulation of pancreatic secretion.

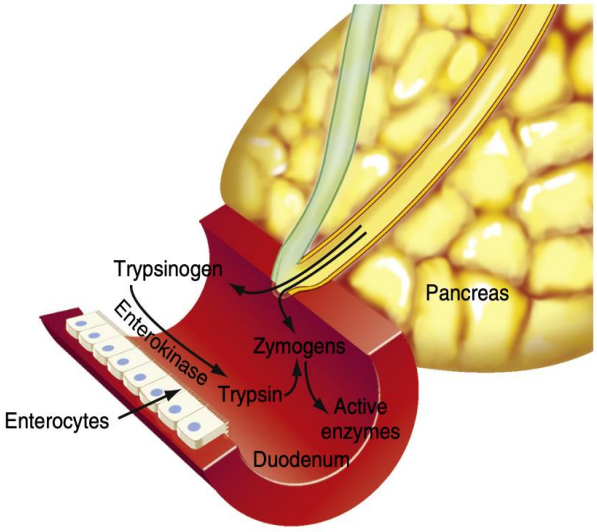


Figure 3*. Inactive proenzymes reaching the duodenum by the pancreatic duct system become activated by enterokinase which is an endopeptidase produced by S-cells in the duodenum.*
From: Sleisenger and Fordtran's Gastrointestinal and Liver Disease 11th edition 2020. With permission from Elsevier.

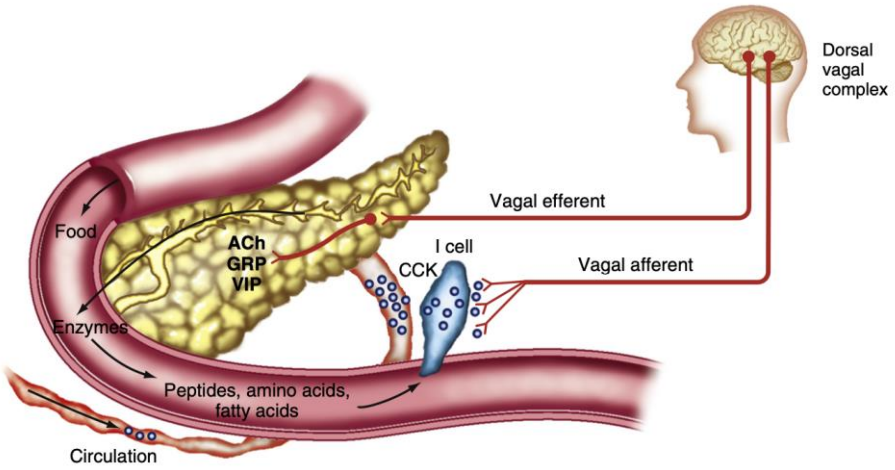


Figure 4*. This figure depicts the complex interplay of the humoral and neural regulation of pancreatic secretion. Food that reaches the duodenum stimulates specialised cells to liberate secretin and cholecystokinin with stimulation of pancreatic secretion. Vagal efferent and afferent loops modulate these processes.*
From: Sleisenger and Fordtran's Gastrointestinal and Liver Disease 11th edition 2020. With permission from Elsevier.

The pancreatic duct cells secrete approximately 1000 ml alkaline and iso-osmotic pancreatic juice every day. Production of a bicarbonate rich fluid is required for buffering of the gastric acid enabling optimal activity of pancreatic enzymes.

The **endocrine pancreas** is represented by the islets of Langerhans comprising five different endocrine cell types (alpha, beta, delta, epsilon, and epsilon). These hormone-producing cells secrete their hormones directly into the blood circulation (glucagon, insulin, somatostatin, ghrelin, and pancreatic polypeptide, respectively). The majority of islets of Langerhans are located in the pancreatic head. (3, 4)

1.2 Measurement of exocrine pancreatic function

A variety of different tests were developed to evaluate exocrine function which can be divided into direct and indirect function tests (***Table 2***). Direct tests are characterized by the sampling of the pancreatic secretion in the duodenum after placement after duodenal intubation and tube placement (by endoscopy or fluoroscopy). After intravenous administration of secretin, the volume and bicarbonate secretion are measured (secretin-test). Following the i.v.

administration of CCK, the duodenal output of pancreatic enzymes (such as amylase, trypsin, lipase or chymotrypsin) is stimulated and can be sampled during different time points (pancreozymin-test).

Test	Description	Advantages	Disadvantages	Clinical Indications
DIRECT				
Secretin	Measurements of volume and HCO_3^- secretion into the duodenum after IV secretin	Provide the most sensitive and specific measurements of exocrine pancreatic function	Require duodenal intubation and IV administration of hormones; not widely available	Detection of mild, moderate, or severe exocrine pancreatic dysfunction
CCK	Measurements of duodenal outputs of amylase, trypsin, chymotrypsin, and/or lipase after IV CCK			
Secretin and CCK	Measurements of volume, HCO_3^-, and enzymes after IV secretin and CCK			
INDIRECT (REQUIRING DUODENAL INTUBATION)				
Lundh test meal	Measurement of duodenal trypsin concentration after oral ingestion of a test meal	Does not require IV administration of hormones	Requires duodenal intubation, a test meal, and normal anatomy, including small intestinal mucosa; not widely available	Detection of moderate or severe exocrine pancreatic dysfunction when a direct test cannot be done (e.g., due to limited availability)
INDIRECT (TUBELESS)				
Fecal fat	Measurement of fat in the stool after ingesting meals with a known amount of fat	Provides a quantitative measurement of steatorrhea	Requires sufficient dietary fat intake and collection of stool; only detects severe pancreatic dysfunction	Detection of severe exocrine pancreatic dysfunction and steatorrhea
Fecal chymotrypsin	Measurement of chymotrypsin or elastase1 in the stool	Do not require IVs, tubes, or administration of oral substrates	Insensitive for detecting mild or moderate dysfunction	Detection of severe exocrine pancreatic dysfunction
FECAL ELASTASE 1				
NBT-PABA	Oral ingestion of NBT-PABA or fluorescein dilaurate with a meal, followed by measurements of PABA or fluorescein in serum or urine	Provide simple measurements for severe pancreatic dysfunction	Do not detect mild or moderate dysfunction; results may be abnormal in patients with small intestinal mucosal disease	Detection of severe exocrine pancreatic dysfunction
Fluorescein dilaurate				

NT-PABA, N-benzoyl-L-tyrosyl-para-aminobenzoic acid.

Table 2: Overview of direct and indirect pancreatic functions tests. *Advantages, disadvantages and clinical indications are noted.*
From: Sleisenger and Fordtran's Gastrointestinal and Liver Disease 11th edition 2020. With permission from Elsevier.

The advantage of direct tests is that direct quantification of pancreatic secretion is possible. A disadvantage is the invasiveness of this test and the lack of commercially available CCK and secretin. Even mild and moderate reductions of stimulated pancreatic secretion can be detected by this test.
The Lundh test is an indirect pancreatic secretory function test that requires duodenal intubation. This test can be performed when secretin or CCK is not available for direct testing as pancreatic secretion is stimulated by a test meal. Duodenal placement of a catheter is still required for aspiration of the pancreatic secrete at different time intervals.

Other indirect tests are non-invasive and involve measuring fecal fat, fecal chymotrypsin of fecal elastase.
Measuring fecal fat is a cumbersome method. Patients have to follow a strictly defined diet when collecting all stool samples for 72 hours. The test is excellent to detect steatorrhea and severe pancreatic insufficiency.
The PABA test was formerly used and after ingestion of N-benzoyl-tryosyl para-aminobenzoic acid (NBT-PABA), PABA can be quantified in the urine. This test is rarely performed nowadays and detects severe pancreatic insufficiency. (5)
The most-widely used test is the fecal elastase test which indicates significantly decreased pancreatic function and which will be explained in the following in more detail.

Quantifying fecal elastase-1 (FE-1) by enzyme-linked immunosorbent assay is currently the most widespread screening test for diagnosing exocrine pancreatic insufficiency (***Figure 5***). In contrast to other pancreatic enzymes is elastase-1 highly stable and is not degraded during the gastrointestinal passage. Generally, a FE-1 concentration below 200 μg/g feces is considered as pathological and compatible with PEI. It is of note, there is disagreement among experts about which FE-1 concentration can be used as a cut-off to diagnose PEI and there are studies suggesting that FE-1 as low as 15 μg/g feces indicates PEI with sufficient certainty. (6) Others have shown that low FE-1 concentrations can provide a false-positive and wrongly indicate PEI in patients with watery diarrhea. These are some of the reasons that more sensitive methods are needed to diagnose PEI and that additional tests are required to interpret mildly reduced FE-1 levels 100-200 μg/g. (7-9)

Figure 5. *The fecal elastase-1 ELISA test.*
From https://www.schebo.com/products/pancreatic-elastase-1/. With permission from ScheBo Pancreatic elastase-1 stool test, Biotech AG, Giessen, Germany.

According to EU guidelines the **^{13}C-mixed triglycerides (^{13}C-MTG) breath test** is an alternative for the elastase-1 test (***Figure 6***).

Vantrappen et al. introduced the ^{13}C-mixed triglycerides (^{13}C-MTG) breath test which is regarded to be more sensitive than FE-1 testing and which specifically detects maldigestion of triglycerides (10). The subjects receive a test meal consisting of bread, butter and ^{13}C-marked triglycerides (2-octanoyl (1-13C)-1,3 distearoyl glycerol) which is a non-radioactive test substance.

In the presence of lipase in the GI tract, triglycerides are metabolized, and ^{13}C-fatty acids are subsequently metabolized in the liver to $^{13}CO_2$. The concentration of $^{13}CO_2$ can be measured at different points in time the exhaled air and correlates directly with the lipase activity. ^{13}C-MTG breath test detects moderate pancreatic exocrine insufficiency noninvasively and reliably. A disadvantage of this test is that it requires prolonged breath sampling (up to 6 hours). Shortening of the ^{13}C -MTG test from 6 to 4 hours of duration was shown to have similar diagnostic accuracy which increased clinical usability. (10, 11)

Figure 6. *In order to calculate the $^{13}CO_2/^{12}CO_2$ isotope ratios in exhaled breath samples infrared spectroscopy was applied (IRIS-3; Wagner Analysen Technik GmbH, Bremen Germany/Kibion Bremen/Sweden*

1.3 Pathophysiology and etiology of chronic pancreatitis

Chronic pancreatitis (CP) is a chronic inflammatory disease of the pancreas with an inflammatory destruction of the parenchyma leading to fibrosis, atrophy and loss of pancreatic function resulting in exocrine and in the later course endocrine insufficiency. The initial steps of chronic pancreatitis are intrapancreatic enzyme activation and acute inflammation. Chronic inflammation with acute exacerbations of pancreatitis ultimately results in severe morphological changes with calcifications, atrophy and changes of the pancreatic duct system. Chronic pancreatitis is a rather rare disease with a prevalence rate of 15.5-52.4 cases per 100,000 inhabitants and an incidence rate around 5 per 100,000 inhabitants per year. (12, 13)

Diagnosing early CP with nonspecific symptoms and lack of marked pancreatic morphologic changes is a challenge and is one of the reasons that CP may be underdiagnosed.

Alcohol and smoking are still the most important etiological factors for chronic pancreatitis, although smoking and alcohol overconsumption is decreasing in many Western countries. All main etiologies are summarized in the M-ANNHEIM classification (alcohol consumption, nicotine, nutritional factors), hereditary (mutations in PRSS1, CFTR, SPINK1, CTRC genes), ductal obstruction and autoimmune factors. (14, 15) Importantly, patients may have a combination of different risk factors resulting in a more rapid progression (e.g. smoking and alcohol and mutations in risk genes).

The key pathophysiological steps vary according to the underlying etiology. In alcoholic pancreatitis, acetaldehyde has direct toxic effects on acinar cells triggering inflammation with subsequent necrosis and apoptosis. Moreover, acetaldehyde activates pancreatic stellate cells (PSC) which produce large amounts of extracellular matrix proteins including collagen. Apart from oxidative stress, non-oxidative metabolites (fatty acid ethyl ester/FAEE) contribute to pancreatic injury. (16)

1.4 Diagnosis and management of chronic pancreatitis

Diagnosis of chronic pancreatitis is frequently based on imaging. Endoscopic ultrasound (EUS), contrast-enhanced computed tomography (CT), magnetic resonance imaging (MRI) have the highest sensitivity rates and are the recommended imaging modalities to diagnose chronic pancreatitis. (17) EUS is regarded as having the highest sensitivity to diagnose CP

and the Rosemont classification was developed to define morphological changes of probable and definite CP. (18-21)
EUS enables the assessment of the pancreas with elastography and can even be used to acquire biopsies of the pancreas (EUS-FNB).(22)
CT is an excellent tool for the detection of pancreatic calcifications whereas MRCP enables the characterization of both the parenchyma and the pancreatic duct system with high resolution. Endoscopic retrograde cholangiopancreatography (ERCP) was the gold-standard to diagnose chronic pancreatitis for decades but is no longer used for mere diagnostic purposes due to risk for post-ERCP pancreatitis (PEP).
The main symptom of chronic pancreatitis is abdominal pain that can be challenging to treat successfully and is significantly affecting the quality of life in this patient group. In patients with stones in pancreatic duct and abdominal pain, endoscopic stone removal with baskets with or without extracorporeal shockwave lithotripsy (ESWL) can be performed. Surgical resection should be considered in patients with chronic pain that is not responding to therapy.
CP patients have a high risk for malnutrition which can be attributed to ongoing alcohol misuse, nausea and obstipation due to chronic opioid medications, stenotic complications in the gastrointestinal tract due to mass forming CP or due to exocrine pancreatic insufficiency with maldigestion and malabsorption. All patients with CP should be screened for malnutrition and PEI according to the EU CP guideline. (17)

1.5 Pathophysiology and etiology of pancreatic exocrine insufficiency (PEI)

Pancreatic exocrine insufficiency (PEI) is defined as markedly reduced activity of pancreatic enzymes in the intestinal lumen resulting in maldigestion. The pancreas has a very high reserve capacity and enzyme activity has to be reduced by 90% until steatorrhea occurs. (23)

This implies that minor reductions of enzyme activity do frequently not result in maldigestion and thus are clinically not relevant. These minor reductions are also termed as pancreatic dysfunction.

PEI with maldigestion has a large range of etiologies and pancreatic and non-pancreatic etiologies are well-described **(*Figure 7*)**.

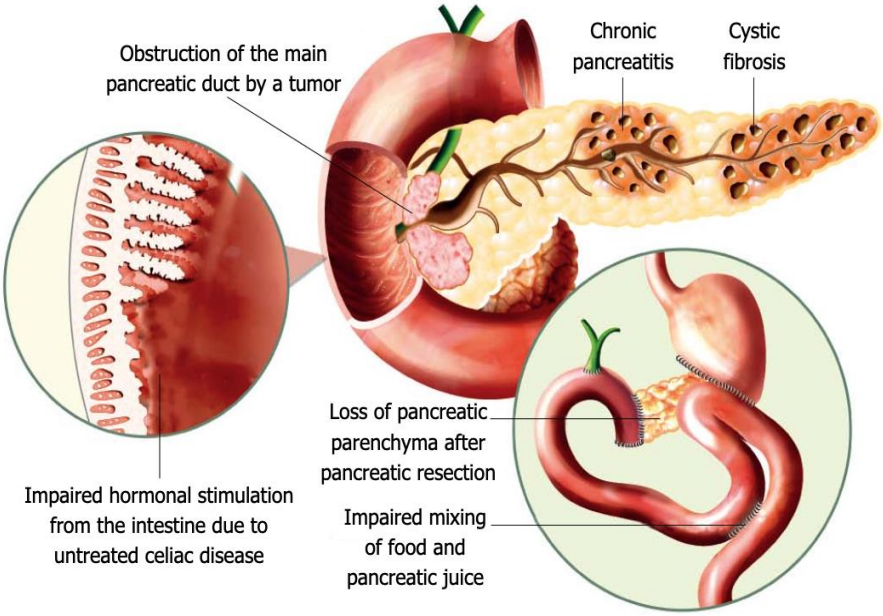


Figure 7. Different causes of pancreatic exocrine insufficiency (PEI).
From: Lindkvist B. Diagnosis and treatment of pancreatic exocrine insufficiency. World J Gastroenterol. 2013 Nov 14;19(42):7258-66. With permission from Baishideng Publishing Group Inc.

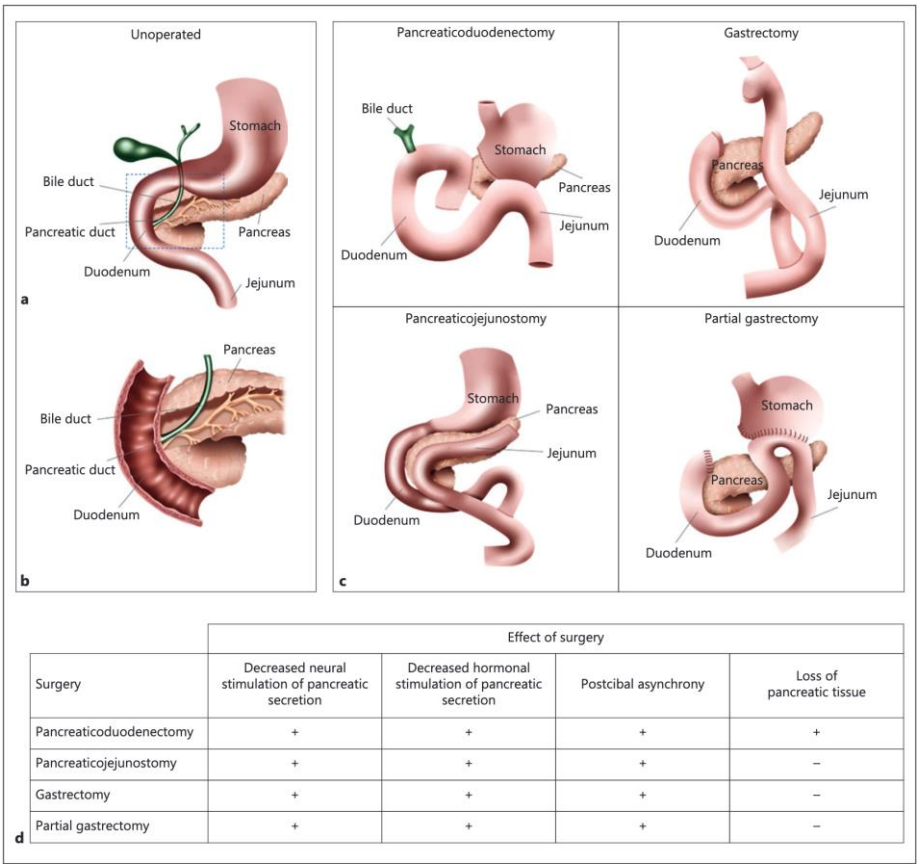
Chronic pancreatitis with atrophy and loss of functional parenchyma is the most frequent cause of PEI. In addition, impaired bicarbonate secretion in CP blunts buffering of gastric acids in the duodenum and reduces lipase activity. In constrast, Zollinger-Ellison syndrome with high acid concentrations in the duodenum with resulting inactivation of pancreatic enzymes is a very rare etiology of PEI. (24-26)

Other causes of PEI are cystic fibrosis with severe pancreatic atrophy due to CFTR mutations and pancreatic resections (e.g. Whipple operation or total pancreatectomy). Benign and malignant pancreatic duct obstructions can impair the excretion of pancreatic enzymes leading to PEI **(*Figure 7*)**. All patients with pancreatic cancer should be treated with PERT according to current guidelines. Even acute pancreatitis can result in a temporary och persistent reduction of pancreatic enzymes. (27)

According to a meta-analysis, the prevalence of PEI was 62% during hospitalisation and 35% during follow-up.

After GI-surgery including bariatric surgery an increased prevalence of PEI was observed (***Figure 8***). Impaired mixing of pancreatic enzymes with ingested food (postcibal

asynchrony), impaired hormonal and neural stimulation of pancreatic secretion contribute to the development of PEI.



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	Effect of surgery			
Surgery	Decreased neural stimulation of pancreatic secretion	Decreased hormonal stimulation of pancreatic secretion	Postcibal asynchrony	Loss of pancreatic tissue
Pancreaticoduodenectomy	+	+	+	+
Pancreaticojejunostomy	+	+	+	–
Gastrectomy	+	+	+	–
Partial gastrectomy	+	+	+	–

Figure 8. Pathophysiology of PEI in common GI surgeries. *Decreased hormonal and neural stimulation of pancreatic secretion, loss of pancreatic tissue and impaired mixing of food and pancreatic enzymes (postcibal asynchrony) are contributing factors of PEI.*
From: Chaudhary A et al. Dig Dis. 2020;38(1):53-68. With permission from Karger AG.

Impaired CCK and secretin production with resultant diminished stimulation of pancreatic secretion is even suggested in patients with active celiac disease. (28, 29)

Atrophy of the pancreas can be observed in patients with chronic pancreatitis but atrophic changes can also be found in patients with type I diabetes mellitus and subjects of old age resulting in PEI. (30, 31)
There is even evidence that patients with IBD or Sjögren´s syndrome have an increased risk of developing pancreatic exocrine insufficiency. (32, 33)

1.6 Treatment of PEI

Treatment of PEI is mandatory and a Spanish study has shown that PEI is associated with an increased mortality (hazard ratio 2.59, 95% CI 1.42–4.71). (34) A meta-analysis has shown that oral supplementation of pancreas enzymes (PERT) improves nutritional status and relieves PEI-related symptoms. Altogether 17 studies with 511 patients were analyzed. PERT improved gastrointestinal symptoms and reduced abdominal pain, reduced fecal fat and normalized nutritional parameters.(35)

There is accumulating evidence that enzyme replacement therapy is important to treat the deficiencies of micro- and macronutrients and thus to decrease the associated risks like osteoporosis even if patients do not have characteristic PEI-related symptoms like steatorrhea. (23, 36, 37)

Enteric-coated mini-microspheres are used for oral pancreatic enzyme replacement therapy (PERT). Pancreatic enzyme preparations are extracted from porcine pancreata. Per meal 40,000-50,000 lipase units of microencapsulated pancreatic enzymes are recommended and 20000-25000 lipase units with every snack. In compliant non-responders the dose can be doubled, or proton-pump inhibitors added. If this strategy does not resolve symptoms other possible underlying diseases should be ruled out. A treatment algorithm was recently published (17).

1.7 Complications of pancreatic exocrine insufficiency

Symptoms of PEI encompass diarrhea with fatty stools, bloating, abdominal pain, malabsorption with reduced calorie intake, deficiencies in micronutrients (calcium, zinc and selenium) and fat-soluble vitamins. (38)

The pancreas as a central digestive gland can be the cause for malabsorption due to pancreatic diseases as a chronic inflammation. From the functional point of view, pancreatic bicarbonate secretion is most important for duodenal neutralization of gastric acid to enable pancreatic enzymes to work optimally. PEI is defined as a critically reduced intestinal pancreatic enzyme activity resulting in steatorrhea, bloating, abdominal discomfort, weight loss, and complications of malnutrition (deficiency in fat-soluble vitamins with consequences like osteoporosis). (24)

Deficiency of fat- soluble vitamins (A, D, E and K) and micronutrients (zinc, magnesium, calcium, thiamine and folic acid) have been demonstrated in patients with CP. (33-37) As the risk of mortality in patients with CP is related mainly to extra-pancreatic causes, such as cardiovascular and respiratory disorders and infections and all these events might be worsened by fat-soluble vitamins deficiencies, malnutrition and vitamin deficiency should be carefully considered during the management of CP patients. (39-41)

Osteoporosis is defined as a systemic bone disease characterised by low bone density and deterioration of bone tissue, with a subsequently increased bone fragility resulting in an increased risk for fractures (***Figure 9***). Dual-energy X-ray absorptiometry (DXA) is the gold standard technique for measuring bone mass. Measurements are usually performed at the femoral neck and lumbar spine. Bone mass measurements are recorded as Z score, reflecting the number of standard deviations (SDs) above or below the mean for an age-matched population (in patients < 50 years of age), or T score, reflecting the number of SDs above or below the mean for a young adult population (in patients > 50 years of age and postmenopausal women). (42)
Several disorders and diseases such as hyperparathyroidism, hyperthyroidism, chronic renal failure, chronic liver disease, gastrointestinal disease and drugs (e.g. glucocorticoids, anticoagulants) were shown to be linked to increased bone loss. Low bone mineral density (BMD) is a common metabolic finding in chronic intestinal disorders associated with diarrhoea and malabsorption and it is also frequent in patients with chronic pancreatitis due to steatorrhea, undernutrition, chronic inflammatory status and alcoholism. (43-45)

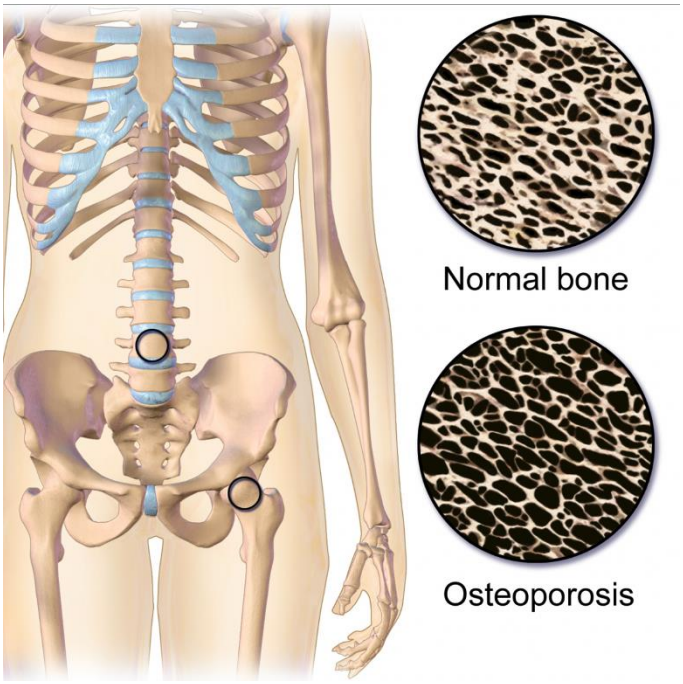

Figure 9. Osteoporosis. *Dual-energy X-ray absorptiometry (DXA) is the gold standard technique for measuring bone mass. Measurements are usually obtained at the femoral neck and lumbar spine. Blausen Medical. Retrieved on 25 January 2016. Wikimedia Foundation. Creative Commons CC BY.*

1.8 IBD and chronic pancreatitis and exocrine pancreatic insufficiency

Inflammatory bowel diseases (IBD) are chronic inflammatory disorders of the gastrointestinal tract encompassing Crohn's disease (CD) and ulcerative colitis (UC). The underlying etiology of the disease involves a combination of genetic, environmental, immunological and gut microbial factors. (46) Mucosal inflammation with diarrhea is characteristic for IBD and is present in approximately 80% of patients. (47)

Pancreatic disorders and abnormalities are not uncommon in IBD. They represent a heterogeneous group of pancreatic manifestations that includes acute pancreatitis (AP), autoimmune pancreatitis (AIP), chronic pancreatitis (CP), imaging abnormalities and asymptomatic elevation of pancreatic enzymes. (48, 49)

In IBD patients most cases of CP are “idiopathic” which can be considered as extraintestinal manifestations of IBD. (50, 51) Other causes are biliary stones or anti-inflammatory drugs used for treating IBD.

Pancreatitis and IBD may have similar clinical signs making it more difficult to make the correct diagnosis in patients with ulcerative colitis and Crohn’s disease. However, elevation of pancreatic enzymes without clinical symptoms and without morphological signs of

pancreatitis should not be considered as pancreatitis. Therefore, only follow-up of pancreatic enzymes is recommended. On the other hand, IBD patients with two of three symptoms of acute pancreatitis (elevantion of pancreatic enzymes/morphological changes compatible with pancreatitis/abdominal pain) should be further investigated and treated. (52, 53)
Chronic pancreatitis associated with UC differs from that observed in CD by the presence of more frequent bile duct involvement, weight loss, and pancreatic duct stenosis. (54)
Pancreatic involvement in CD can be caused by changes in main pancreatic duct, decreased of hormonal signaling due to mucosal inflammation and as a part of autoimmune extraintestinal manifestations. (52)

IBD-related CP appears to have different phenotype compared to alcohol-related chronic pancreatitis with calcifications. Typically, pseudocysts and pancreatic calcifications are more frequently observed in alcohol-related CP compared to IBD-related CP. (52, 53)

1.9 Sjögren's syndrome and pancreatic exocrine insufficiency

Sjögren's syndrome (SS) has been defined as an autoimmune disorder characterized by lymphocytic infiltration of salivary and lacrimal glands, leading to dry mouth (xerostomia) and eye dryness (keratoconjunctivitis sicca). Moreover, a third of the patients with this disease develop several systemic complications, such as renal, pulmonary or neurological manifestations (***Figure 10***). Around 5% of patients with primary SS will develop B-cell lymphoma, which are characteristically found in the most affected organs (e.g. salivary glands). (55) SS can be seen alone (primary SS) or in association with other connective tissue diseases. (56) The estimated prevalence of primary Sjögren's syndrome (pSS) is about 0.2% in the adult population and a yearly incidence of 4/100.000. The reported male/female ratio lies in the range of 1:9 with an onset age of approximately 45 years. (57)

Both the pancreas and salivary glands share important functional and histological similarities. Both produce bicarbonate-rich fluid with digestive enzymes which are secreted into the gastrointestinal tract. Isolated cases of SS in combination with pancreatic calcifications, pancreatic autoantibodies and increased pancreatic enzyme levels have been described in previous studies. (58-61) Pancreatic dysfunction in SS has been hypothesized, but is still a matter of debate. (62)

Pancreatic exocrine insufficiency has been also estimated using the N-benzoyl-L-tyrosyl para-amino-benzoic acid test (NBT-PABA test) and by measuring the trypsinemia by radio-immunoassay in SS. The NBT-PABA test was pathological in 37.5% of SS but in none of the controls and importantly paitients were asymptomatic. Trypsinemia was found to be high in 6.2% of SS. (63) In a similar study PEI was found in 63% of patients. (64)
Afzelius et al examined the morphology, the exocrine and endocrine functions of the pancreas in SS, but without known pancreatic disease, using secretin-stimulated magnetic resonance cholangiopancreatography (MRCP), a Lundh test, oral glucose tolerance test, and blood sampling. The prevalence of pancreatic dysfunction was increased to 25–33% in the study population. (65)

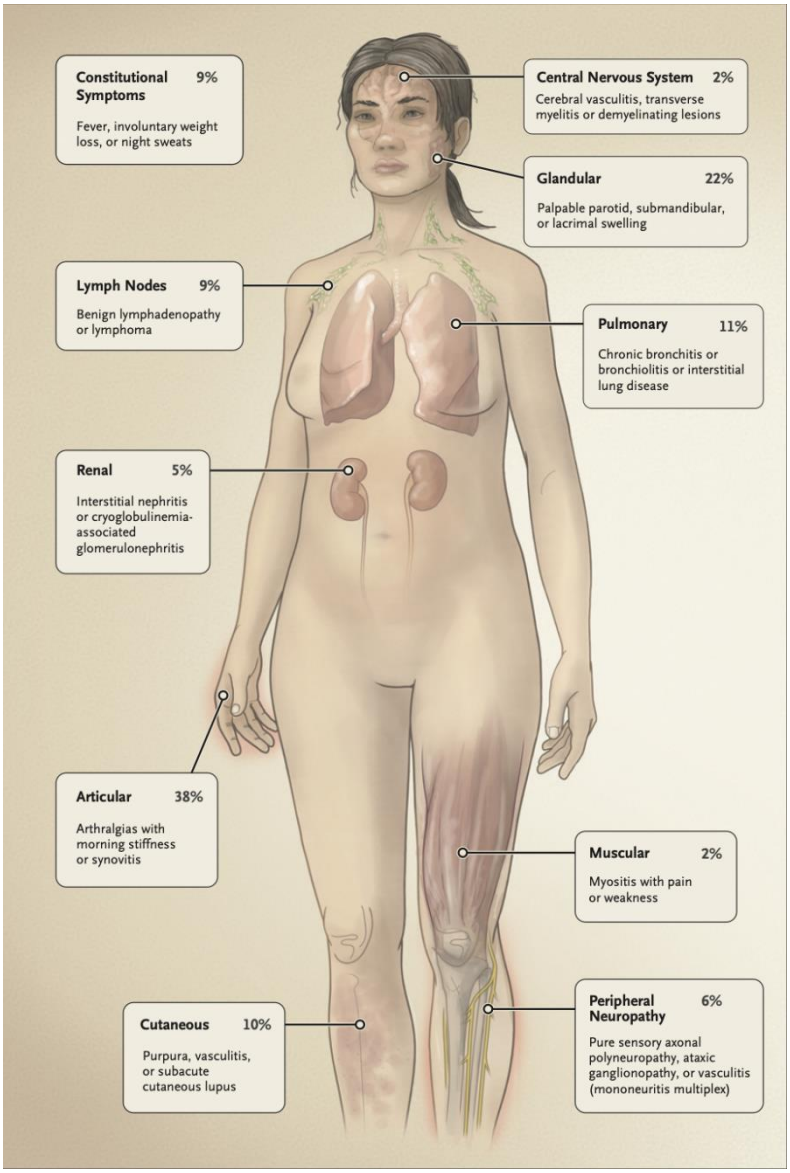

Figure 10. Systemic manifestations of primary Sjögren's syndrome.
The percentages of patients with the various manifestations of primary Sjögren's syndrome were derived from reports in the Sjögren Big Data project,4 which includes information on more than 10,000 patients with primary Sjögren's syndrome from 22 countries.
From: Mariette X, Criswell LA. Primary Sjögren's Syndrome. N Engl J Med. 2018;378(10):931-939. With permission

2. AIMS

The overall aims of this study were to provide a better definition of the risk for complications of pancreatic exocrine insufficiency in patients with chronic pancreatitis and to further elucidate whether diseases such as Crohn's disease and Sjögren´s syndrome are associated with pancreatic exocrine insufficiency.

The specific aims of this study were:

Aim I: To evaluate the prevalence of deficiencies of fat-soluble vitamins in patients with chronic pancreatitis by applying a systematic meta-analysis.

Aim II: To evaluate the prevalence of osteopenia and osteoporosis in patients with chronic pancreatitis and to investigate if vitamin D, CP features and pancreatic exocrine insufficiency (PEI) are risk factors for osteopathy in patients with chronic pancreatitis.

Aim III: To evaluate the prevalence of PEI in patients with Crohn's disease (CD).

Aim IV: To evaluate the prevalence of PEI and gastrointestinal symptoms in patients with primary Sjögren´s syndrome (SS).

3. METHODS

3.1. Paper I

3.1.1 Study design

The study was planned and designed by candidates of a European postgraduate program (Pancreas 2000: https://www.pancreas2000.org/) with members from six different countries, namely Estonia, Germany, Italy, Poland, Spain, Sweden.

The protocol followed the guidelines according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement and the study was registered in the Prospero database (www.crd.york.ac.uk/PROSPERO/). (66)

All studies including specific search terms (such as vitamin A, D, E, K and CP, PEI) were retrieved by MEDLINE. Studies from October 1982 until October 2015 were included. Three reviewers selected independently all studies meeting the inclusion criteria. All data was saved in Microsoft Excel sheets and incongruencies were discussed with a fourth reviewer.

3.1.2 Inclusion and exclusion criteria

The following criteria were used for inclusion: patients with a diagnosis of CP and > 18 years of age, inclusion of at least one of the four fat-soluble vitamins, either a cross-sectional or case-control study design and only full papers in English language.
Case reports or small (<10 patients) case series or publications including patients with other gastrointestinal disease-causing malabsorption were excluded.

3.1.3 Study extraction and quality assessment

After the selection of studies, the following clinical data were extracted: study setting, study design, year of publication, number of patients, gender, age, study period, rate of deficiency of fat-soluble vitamins and normal values, CP characteristics, prevalence of PEI, diabetes and treatment of PEI and source of controls. The quality of the studies was assessed according to the Newcastle-Ottawa scale (NOS) (67).
http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp

NOS serves also as a tool to determine the risk for bias in observational studies and is recommended by the Cochrane Collaboration. (68, 69)

3.2 Paper II

3.2.1 Patients and study design

This multicenter cross-sectional study included patients with chronic pancreatitis from seven European countries (Estonia, Germany, Italy, Poland, Spain, Sweden and the United Kingdom) respectively. Patients were recruited from April 2015 to October 2016. The study was developed from members of an European pancreatology postgraduate program (Pancreas 2000; https://www.pancreas2000.org/).

3.2.2 Inclusion and exclusion criteria

The following inclusion criteria were applied: age >18 and a diagnosis of chronic pancreatitis according to the M-ANNHEIM criteria. (14) The M-ANNHEIM classification of chronic pancreatitis enables classification of patients according to etiology and severity of CP. The classification is well-established, frequently applied and recommended by a a recent European CP guideline. (15, 17)

The following exclusion criteria were used: patients with a diagnosis of pancreatic cancer, liver cirrhosis, chronic renal failure, primary hyperparathyroidism, celiac disease, IBD and previous GI surgery.

3.2.3 Questionnaire

A standardized questionnaire based on the local language of the respective country was used to evaluate a large range of parameters comprising epidemiological data, current drug medication, risk factors for osteopathy, diet, lifestyle factors, past medical history including previous fractures.

3.2.4 Dual energy x-ray absorptiometry (DXA)

Bone mineral density (BMD) of the lumbar spine and of the left femoral neck was assessed by standardized osteodensitometry applying dual energy x-ray absorptiometry (DXA). Results of bone mass measurements were recorded and summarized by T-scores, that reflect the number of standard deviations above or below the mean for a young adult population. Osteopenia is

defined as a T-score < -1 and osteoporosis is defined as a T-score < -2.5. DXA examinations were performed at each participating centre not later than 6 months from the time of enrolment. DXA was performed at the Department for Endocrinology at Karolinska University Hospital, Huddinge.

3.2.5 Laboratory testing

All studied laboratory parameters - apart from vitamin K - were determined at the local study centers. These parameters were: vitamin D, albumin, total and ionized calcium, magnesium, parathormone (PTH), triglycerides, cholesterol, transferrin, hemoglobin, mean corpuscular volume (MCV), lymphocytes, vitamin B12, folic acid, prothrombin time, INR and partial thromboplastin time.
Vitamin K levels were quantified at the laboratory of S. Andrea Hospital, University "Sapienza" Rome, Italy for standardized measurements. Blood from patients of all centers was drawn, immediately thereafter centrifuged and frozen at – 80°C. Finally, frozen samples were shipped to Rome for vitamin K analysis by high performance liquid chromatography (HPLC).

The presence of PEI was investigated by a faecal elastase-1 test. A monoclonal enzyme-linked immunosorbent assay was used to investigate faecal elastase-1 levels (ScheBo Pancreatic elastase-1 stool test, Biotech AG, Giessen, Germany). Results were defined as normal pancreatic function (> 200 mcg/g of stool), mild pancreatic exocrine insufficiency (100 - 200 mcg/g of stool) and severe pancreatic exocrine insufficiency (< 100 mcg/g of stool).

3.3 Paper III

3.3.1 Patients and study design

A monocenter cross-sectional pilot study on CD patients who were recruited at the Department for Gastroenterology at Karolinska University Hospital in Stockholm, Sweden. Data as gender, age, duration of CD and its treatment, smoking, and stool assessment by Bristol Scale were analyzed. (70)

3.3.2 Inclusion and exclusion criteria

Inclusion criteria was: age >18 and a diagnosis of CD. Exclusion criteria were the following: participants who signed informed consent but could not perform FE-1 and participants who could not speak either Swedish or English.

3.3.3 Laboratory testing

PEI was estimated with faecal elastase-1, measured using a monoclonal enzyme-linked immunosorbent assay. Results were classified as normal pancreatic function (more than 200 mcg/g of stool), mild pancreatic exocrine insufficiency (100 to 200 mcg/g of stool) and severe pancreatic exocrine insufficiency (less than 100 mcg/g of stool).(7) Retrospectively we assessed a Calprotectin. (80) Calprotectin is a fecal marker that grades the lever of CD inflammation by measuring a protein released in stool. A normal value is considered by less than 50 μg/g. (71)

3.3.4 Endoscopic activity scores in patients with Crohn´s disease

Recent colonoscopy examinations were retrospectively assessed to rule out active inflammatory bowel disease in the colon. (70, 72) Activity was classified according to the Simple Endoscopic Score of CD (SES-CD) and the Rutgers's Score (RS) in patients after ileocecal resection, respectively. (69, 72, 73) The SES-CD is a simple and validated endoscopic score for describing ileocolonoscopy findings in CD using parameters (ulcer size, ulcerated surfaces and narrowing) scored at each colonic segment from 0 to 3. (73)

The RS scoring includes lesions from the bowel segment: i0: no lesions; i1: < 5 aphthous lesions; i2: > or equal to 5 aphthous lesions with normal mucosa between lesions or skip areas of larger lesions or lesions; i3: diffuse aphthous ileitis with diffusely inflamed mucosa and i4: diffuse inflammation with larger ulcers, nodules and/or narrowing. (72)

3.4 Paper IV

3.4.1 Patients and study design

A monocenter cross-sectional pilot study on SS patients who were recruited from the Department for Rheumatology at Karolinska University Hospital in Stockholm, Sweden. Data

as gender, age, duration of SS, lip biopsies and presence of anti-SSA autoantibodies were analyzed.

3.4.2 Inclusion and exclusion criteria

All male and female patients with primary SS, and all patients older than 18 years of age or younger than 75 years of age were included into the study. The following patients were excluded: patients with secondary SS and all SS patients <18 years of age or > 75 years of age. In addition, all patients with a known pancreatic disease were excluded (e.g. acute/chronic pancreatitis, previous pancreatic diagnostic or therapeutic interventions including surgery and ERCP).

3.4.3 Evaluation of gastrointestinal symptoms

To evaluate gastrointestinal symptoms in SS patients we applied the Gastrointestinal Symptom Rating Scale (GSRS), which represents a reliable, and strictly validated questionnaire comprising 15 items with five groups of symptoms comprising reflux, abdominal pain, constipation, diarrhea and indigestion. (74-76). Sex and age-matched controls were chosen from the previously described *PopCol* study, which represents a population-based colonoscopy study.

We have evaluated gastrointestinal symptoms through an established survey Gastrointestinal Symptom Rating Scale (GSRS-IBS). GSRS-IBS contains 13 items which measure severity of gastrointestinal symptoms such as abdominal pain, pain relieved by a bowel action, bloating, passing gas, constipation, diarrhea, loose stools, hard stools, urgent need for bowel movement, incomplete bowel emptying, fullness shortly after meal, fullness long after eating, and visible distension. (77) The study was based on a Swedish population sample of 1158 randomly selected participants provided data on the GSRS-IBS. (78, 79)

3.4.4 ^{13}C-mixed triglyceride breath test

PEI was assessed with a ^{13}C-mixed triglyceride breath test. (13) ^{13}C-MTG breath test was performed at Karolinska University Hospital in 21 patients. Results were considered as normal in patients with a cumulative ^{13}C exhalation > 20.9% after 5 hours of testing according to Keller et al. (11)

Our test meal consisted of two slices of white bread, 20 g of butter, and 30 g of chocolate cream (Nutella; Ferrero, Germany). The latter was carefully mixed with 200 mg of ^{13}C-MTG (^{13}C- MTG: [2-octanoyl(1-^{13}C)-1,3 distearoyl glycerol]; Euriso-Top, catalogue number INC650P, triglyceride with ^{13}C-octanoic acid [labelled with 1 ^{13}C-atom] bound to the Sn-2 position and unmarked long-chain fatty acids bound to Sn-1 and Sn-3 positions).
The meal was ingested together with 200 mL of water within 10 minutes. The total caloric value was 420 kcal (1770 kJ). Breath samples were taken before the ingestion of the test meal and every 30 minutes for 5 hours thereafter. No physical activity was allowed throughout the test. The $^{13}CO_2/^{12}CO_2$ isotope ratio in breath was determined using isotope-selective non-dispersive infrared spectrometry (IRIS-3; Wagner Analysen Technik GmbH, Bremen Germany/Kibion Uppsala/Sweden). The results were analyzed as % values and expressed as a maximal or cumulative percentage of dose of ^{13}C recovered over 1- to 5-hour intervals.

3.4.5 Laboratory testing

PEI was estimated with faecal elastase-1, measured using a monoclonal enzyme-linked immunosorbent assay. Results were classified as normal pancreatic function (more than 200 mcg/g of stool), mild pancreatic exocrine insufficiency (100 to 200 mcg/g of stool) and severe pancreatic exocrine insufficiency (less than 100 mcg/g of stool). (7)

3.5 STATISTICAL ANALYSIS

3.5.1 Paper I

Deficiency rates of all studied fat-soluble vitamins were pooled on the basis of all included studies (cohort studies and case-control studies). For case-control studies, deficiency rates in CP patients and controls were separately pooled, and the individual relative odds ratio (OR) and 95% confidence interval (CI) calculated.

The software package Comprehensive Meta-Analysis (Biostat, Englewood, NJ, USA) was used for all statistical calculations. The quantity of heterogeneity was assessed by means of the I^2 values. (80) We considered an I^2 value of 25% or lower as low heterogeneity, and an I^2 value of 75% or higher as considerable heterogeneity.

The Begg and Mazumdar test was used to assess publication bias. A p-value < 0.05 regarded as statistically significant.

3.5.2 Paper II

Fisher exact test was used for analyzing categorical variables and t-test for continuous variables.

A univariate and a multivariate logistic regression analysis was performed to identify nutritional and clinical parameters that were correlated with BMD. For every parameter the odds ratio (OR) and the respective 95% confidence interval (CI) was calculated. The possible correlation between continuous variables was assessed by a Pearson correlation test. All p-values were two-sided and a p-value < 0.05 were regarded as statistically significant. All statistical analyses were carried out by MedCalc version 13 (MedCalc Software, Belgium).

3.5.3 Paper III

Standard methods for descriptive statistics were used. Categorical variables were presented as frequencies (n) and percentages (%). Continuous variables were presented by median and range.

3.5.4 Paper IV

Descriptive statistics were used to summarize patient demographics (gender, age), disease characteristics (duration of SS, lip biopsy findings and presence of autoantibodies), and outcomes. Categorical variables are presented by frequency (N) and percentages (%). The

differences between those with Sjögren´s syndrome and those without were evaluated by chi-square tests for these variables. Mean ± standard deviation (SD) or median (interquartile range) was used to illustrate continuous variables. T tests were used to estimate the variations for such factors.

Moreover, we performed a propensity score-matched analysis to create comparable risk groups with respect to 11 different items of GI symptoms in SS patients and sex and age-matched controls from the PopCol study. Matching was achieved using propensity scores, which represent the probability of group assignment conditional on observed baseline covariates. (81) Matching pairs were formed in a manner to ensure that matched subjects had similar values of the propensity scores i.e. for a patient with Sjögren´s syndrome. All the control subjects whose propensity score lay within a specified distance (caliper distance) were identified. A smaller distance ensures a better chance to have similar groups with respect to the set of covariates, and thus we chose a caliper distance of 0.0001. Participants with propensity scores outside the specified caliper distance of the propensity score were removed from the matching sample. Participants were matched for any significant differences seen between the two groups with respect to age and gender. Standardized differences were calculated to compare patient features before and after matching with imbalance being defined as an absolute value greater than 0.10 (small effect size). (81)

Matching was performed using the nearest neighbor algorithm based on a four-digit match of the propensity score. This strategy allowed the inclusion of comparable cohorts with respect to different items of GI symptoms. Statistical analyses were performed in R Statistical software version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria) using the package MatchIt.

Univariate Logistic Regression Analysis: After propensity score matching, we then conducted univariate logistic regression analyses.

3.6 ETHICAL CONSIDERATIONS

For the first study we did not need ethical approval because it was meta-analysis. The second, third and fourth studies were approved by the Regional Review Board, Stockholm, Sweden. (EPN Dnr :2016/491-31/2 and Dnr: 2017/650-31/4) Oral and written informed consent was obtained from all patients.

4. RESULTS

4.1 Paper I

Initially, 381 potential studies were retrieved from PUBMED and ultimately 12 articles with altogether 548 patients with chronic pancreatitis met the inclusion criteria for further meta-analysis. Five cross-sectional studies and seven case-control studies were included for further analysis that were published from October 1982 until October 2015 (***Figure 11***).

The overall rate of deficiencies for vitamin D, E and A were 57.6% (95% CI, 43.9-70.4), 29.2% (95% CI, 8.6-64.5) and 16.8% (95% CI, 6.9-35.7), respectively.
Only one study investigated vitamin K deficiency in patients with chronic pancreatitis.

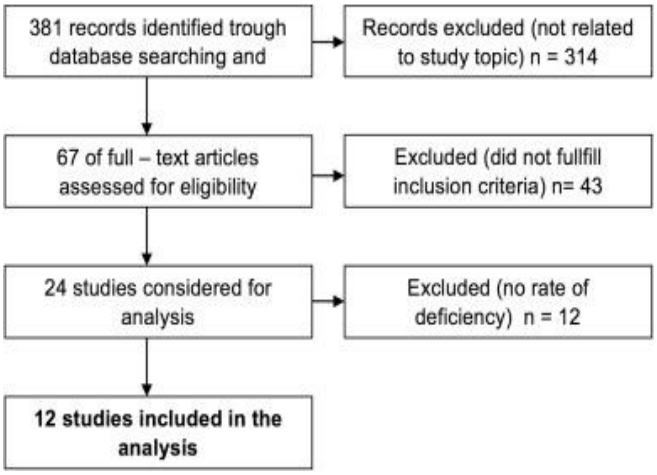

***Figure 11**. **The selection process of suitable publications** is illustrated as a PRISMA diagram (http://www.prisma-statement.org/).*

The large majority of the included studies were conducted in Europe (7/12), two studies were from India and only one study from each of the following countries: South Africa, Argentina, and the US, respectively.
Alcohol was the most frequent etiology for chronic pancreatitis, followed by idiopathic chronic pancreatitis. Two studies did not note the etiologic factor.
The prevalence of PEI had a high variation ranging from 35% to 100%. In parallel, rates of patients receiving oral supplementation of pancreatic enzymes ranged from 46% to 100%.

Results of the meta-analysis are illustrated as forest plots.

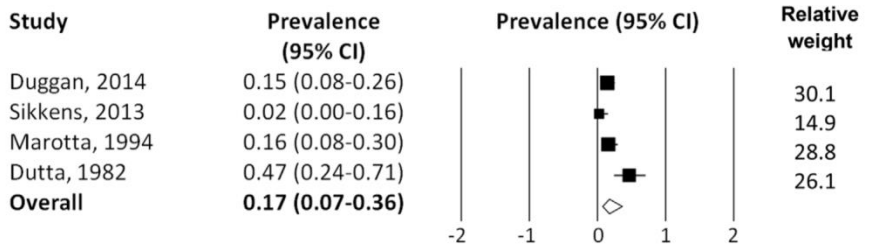

Figure 12. *The pooled prevalence vitamin A deficiency in chronic pancreatitis was 16.8% (95% CI, 6.9%-35.7).*

Four studies (40, 82-84) with altogether 161 patients were included in this analysis and showed a pooled prevalence rate of 16.8% for Vitamin A deficiency in 161 patients with chronic pancreatitis. When two studies with lower quality was excluded the pooled prevalence rate was 7.7% (95% CI 1.4-33.5). Of note, a considerable heterogeneity between the studies was observed (I^2=75%) (***Figure 12***).

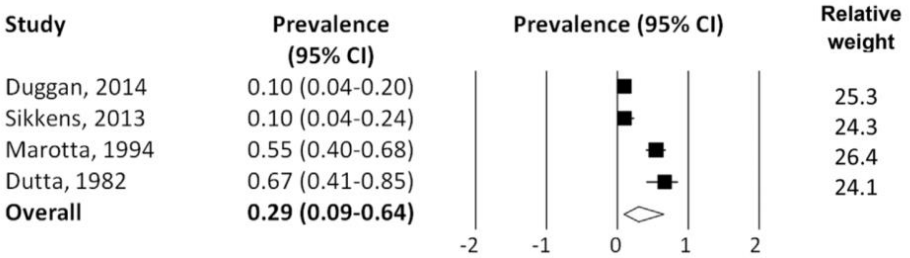

Figure 13. *The pooled prevalence of vitamin E deficiency in chronic pancreatitis was 29.2% (95% CI, 8.6%-64.5).*

This calculation was based on four studies (40, 45, 83, 84) including 161 patients. A publication bias was ruled out by testing according to Begg and Mazudmar. Heterogeneity was considerable (I^2=92%).

The prevalence of vitamin E deficiency was much lower, when only the two high quality studies (40, 84) were included in the analysis (9.8%, 95% CI, 5.4-17.3) (***Figure 13***).

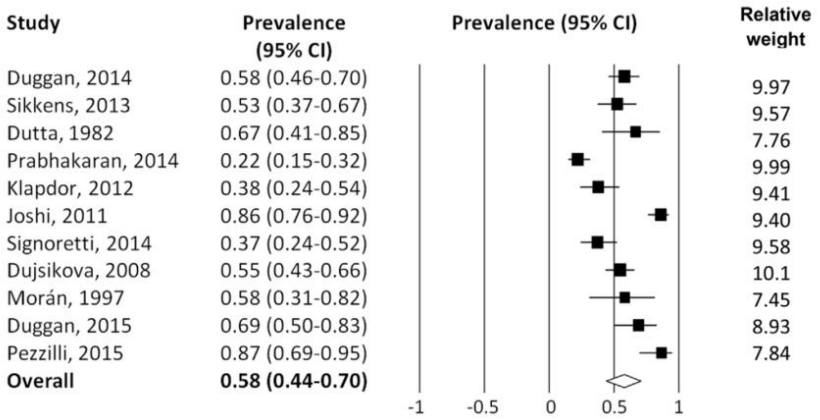

Figure 14*. The pooled prevalence of vitamin D deficiency in chronic pancreatitis was 57.6% (95% CI, 43.9-70.1).*

Eleven studies with 504 CP patients were included. The pooled prevalence of deficiency was 57.6% (95% CI, 43.9-70.4%) (***Figure 14***). Different definitions of vitamin D deficiency according to different cut-off values were taken into account. Vitamin D < 20 ng/ml was regarded as deficiency.

Studies with high rates of PEI (>70%) (40, 43, 84, 85) were compared with studies with lower rates of PEI. (94-96) Interestingly, studies with high rates of PEI demonstrated only a minor increase in the rate of vitamin D insufficiency.

Only five studies (82, 85-88) were available to compare the prevalence of vitamin D deficiency in patients with chronic pancreatitis versus control. No statistical difference was found (OR 1.17; 95% CI, 0.77-1.78; p=0.46).

4.2 Paper II

Altogether, 211 patients with CP were included in study. Roughly two-thirds of patients (67%) were male and the mean of age of the cohort was 60 years. Alcohol was the etiology in 43.6% of cases representing the main etiology of CP in this cohort.
More than two-thirds (69%; 145/211) had a history of smoking and 70% of them were considered as heavy smokers. Diabetes mellitus was present in 37% (77/211) of cases. The mean BMI at the time of enrolment was 24 ± 4. In total, 69 female CP patients were included and almost every third female patient (29%) was in menopause.

According to the M-ANNHEIM classification, 18% had advanced CP and more than half of CP patients (56.42%) had PEI.

In total, more than half of patients had a vitamin D deficiency (56.4% (115/204)) and almost one-third of patients had had vitamin K deficiency (31.5% (56/178)). All patient characteristics are summarized in (**Table 3).**

Table 3: Main characteristics of all patients with chronic pancreatitis (n=211)

Patient' s features	**Number (%)**
Sex	
Male	142/211 (67.29%)
Female pre-menopause	8/211 (3.79%)
Female post-menopause	61/211 (28.90%)
Body mass index (meaan)	24 ± 4
Diabetes	77/211 (37%)
Ever alcohol drinkers	127/211 (60%)
Ever smokers	145/211 (69%)
Aetiology	
Alcoholic	92/211 (43.60%)
Idiopathic	40/211 (18.95%)
Hereditary	9/211 (4.26%)
Obstructive	12/211 (5.68%)
Other	58/211 (27.48%)
Disease Severity	
Minor	74/211 (35%)
Increased	99/211 (47%)
Advanced	32/211 (15%)
Marked	6/211 (3%)
Pancreatic Exocrine Function	
Normal	78/179 (43.57%)
Faecal elastase < 200 mcg/g > 100 mcg/g	29/179 (16.20%)
Faecal elastase < 100 mcg/g	72/179 (40.22%)
Pancreatic enzyme replacement therapy	116/211 (54.97%)
Bone mineral density	
Normal	76/211 (36%)
Osteopenia	89/211 (42.18%)
Osteoporosis	46/211 (21.80%)
Vitamins dosage	
Vitamin D (mean value; ng/ml)	20.2 ± 12
Vitamin D deficiency (< 20 ng/ml)	119/211 (56.39%)
Vitamin K (mean value; ng/ml)	0.64 ± 0.9
Vitamin K deficiency (< 0.2 ng/ml)	56/178 (31.46%)

Data are presented as rate or as mean (± SD)

An univariate and multivariate regression analysis was conducted to identify possible risk factors for osteopathy on the basis of all 211 CP patients. Only three parameters were identified as potential risk factors both in the univariate and the multivate analysis (***Table 4***).

Table 4: Possible risk factors for osteoporosis identified by multivariate logistic regression analysis in all CP patients (n=211). Only statistically significant results are shown.

CP (n=211)	*OR (95% CI)*	*P-value*
Female sex	**2.78** (1.02–1.11)	0.001
Age	1.07 (1.16–6.68)	0.021
BMI	0.84 (0.74–0.95)	0.006

Based on both the univariate and multivariate analysis, the following parameters were not associated with osteopathy: alcohol as the etiologic factor, smoking, severity of CP, presence of PEI, presence of oral enzyme supplementation (PERT), vitamin K and D deficiency.

In a separate Pearson correlation test, no statistically significant correlation between vitamin D levels and T-scores could be demonstrated.

A separate univariate and multivariate analysis was conducted to study the correlation of vitamin D and K only in male patients. Interestingly, vitamin K deficiency in patients with osteoporosis (27.7%) was more frequent compared with those without osteoporosis (5.9%) (OR 95% CI, 1.31-21-40; p=0.01).

4.3 Paper III

Twenty patients were included in this study comprising 65% (13/20) males and 35% (7/20) females. Mean duration of CD was 15.7 ± 2.1 years. Eleven patients (55%) did not have a history of bowel surgery and 9 (45%) patients underwent ileocecal resection. FE-1 was normal in all patients. The majority of patients 16 (80%) were non-smokers and alcohol overconsumption was not reported. None of the patients had a previous history of pancreatitis.

Most patients had an ongoing treatment with one anti-inflammatory drug and the majority of patients received oral azathioprine (8/20) (**Table 5**).

Table 5: Demographic and clinical characteristics of all included patients

N	GENDER	AGE (years)	DURATION OF CD (years)	SMOKING	CLINICAL STATUS	ENDOSCOPIC STATUS	CALPRO	TREATMENT OF CD	FE-1 (µg/g)
1	male	60	10	former	stool 3x per day (Bristol 4-6)	status post ileocecal resection Rutgeerts score: i2	31	azathioprine	>500
2	male	48	14	never	stool 4x per day (Bristol 4-6)	SES-CD:2	20	5-ASA	>500
3	male	45	5	never	stool 4x per day (Bristol 4-6)	SES-CD:0	43	azathioprine	>500
4	male	44	12	former	stool 2x per day (Bristol 4)	SES-CD: 0	346	azathioprine	>500
5	female	57	1	never	stool 5-7x per day (Bristol 6-7)	status post ileocecal resection Rutgeerts score: i2	320	prednisolone and tacrolimus (status post liver transplantation)	>500
6	female	57	30	former	stool 1-2x per day (Bristol 4)	SES-CD: 0	45	azathioprine and 5-ASA	>500
7	male	48	17	never	stool 1-2x per day (Bristol 4)	SES-CD: 0	306	infliximab	>500
8	male	54	29	never	stool 1x per day (Bristol 4)	status post ileocecal resection Rutgeerts score: i0	16	prednisolone	>500
9	male	30	5	never	stool 2x per day (Bristol 6)	SES-CD: 6	65	azathioprine	>500
10	male	52	13	never	stool 2x per day (Bristol 4)	status post ileocecal resection Rutgeerts score: i4	123	azathioprine	>500
11	male	43	17	never	stool 2x per day (Bristol 4)	SES-CD: 0	141	azathioprine	>500
12	male	50	40	never	stool 4-5 x per day (Bristol 6)	status post 2x operations of small bowel with removal of 75 cm of small bowel Rutgeerts score i4	103	5-ASA	>500

13	female	51	1	former	stool 1-6x per day (Bristol 4-7)	status post ileocecal resection Rutgeerts score: i2	109	azathioprine	338
14	female	42	26	never	stool 1-2x per day (Bristol 4-6)	status post ileocecal resection Rutgeerts score: i0	15	no regular treatment after surgery	>500
15	female	67	1	never	stool 1-2x per day (Bristol 5-6)	SES-CD: 0	125	no regular treatment	246
16	male	29	20	never	stool 1-2x per day (Bristol 4-5)	SES-CD: 0	41	5-ASA	>500
17	male	56	35	never	stool 1x per day (Bristol 4-5)	SES-CD: 0	16	adalimumab (due to rheumatological disease)	352
18	female	29	13	never	stool 2x per day (Bristol 4-6)	status post ileocecal resection Rutgeerts score: i4	75	azathioprine	>500
19	male	47	7	never	stool 4-5x per day (Bristol 6)	status post ileocecal resection Rutgeerts score: i1	73	budezonide	411
20	female	58	10	never	stool 2x per day (Bristol 5-6)	SES-CD: 0	67	adalimumab	434

CD=Crohn's disease; calpro=calprotectin; FE-1=fecal elastase-1; SES-CD=Simple Endoscopic score of Crohn's Disease; 5-ASA=5-acetyl salicylic acid

4.4 Paper IV

One-hundred-twenty-two patients were contacted by mail and have been invited to participate in the study. Altogether, 57 SS patients responded and were included in the study. A characteristic feature of primary SS is that primarily females are affected. Consequently, 92 % of included patients were females with a median age of 63 years and a median age of diagnosis of 52 years (**Table 6 and 7**). Almost all patients (96%) had undergone lip biopsy and 44% had positive SS-A autoantibodies. All patients met the classification criteria of the American-European Consensus Group (AECG). (89)

Fifty of fifty-seven SS patients (87%) provided a fecal sample for FE-1 testing and in all tested patients FE-1 was normal. All patients were invited to perform a ^{13}C-MTG breath test

and 37% (27/57) of SS patients gave their approval to conduct a breath test which was normal in all (data not shown).

Almost all SS patients responded to the GSRS questionnaire 53/57 (93%). We compared results of 42 SS patients with results of 82 sex and age-matched controls stemming from the PopCol study and calculated the odds ratios of every item in a univariate logistic regression analysis (**Table 8**).

A large number of patients noted moderate to severe symptoms such as abdominal pain, bloating, diarrhea, loose stools, hard stools, or constipation. Interestingly, symptoms closely related to IBS were frequently noted (feeling of incomplete emptying after defecation; relief of abdominal pain after bowel movement).

Based on the univariate logistic regression analysis, the highest odd ratios were noted for the following moderate to severe symptoms: bloating (OR: 27.9, 95% CI: 9.81-91.9), feeling of incomplete emptied bowel after defecation (OR 21.4, 95%: 6.95-75.8), and abdominal pain relieved by bowel action (OR 17.8, 95% CI: 6.04-62.2).

Table 6. Patient characteristics of patients with Sjögren´s syndrome (n=57)

Characteristic	**N = 57**[1]
Age	63 (10)
Age at diagnosis	52 (11)
Positive biopsy (labial gland)	
yes	50 (96%)
no	2 (3.8%)
Positive anti-SSA	
yes	25 (44%)
no	32 (56%)

[1]Statistics presented: mean (SD); n (%)

Table 7. GI symptoms in Sjögren´s syndrome (n=42) compared to age and sex-matched healthy controls (n=82)

Characteristic	Sjögren`s patients	Controls	p-value
age	61 (54-68)	61 (54-67)	0.9
gender			>0.9
Males	3 (7.1%)	6 (7.3%)	
Females	39 (93%)	76 (93%)	
Q 1. Abdominal pain			<0.001
No Symptoms	8 (19%)	47 (57%)	
Minor/mild	12 (29%)	27 (33%)	
Moderate to severe	22 (52%)	8 (9.8%)	
Q 2. Abdominal pain relieved by bowel action			<0.001
No Symptoms	13 (31%)	58 (71%)	
Minor/mild	9 (21%)	19 (23%)	
Moderate to severe	20 (48%)	5 (6.1%)	
Q 3. Bloating			<0.001
No Symptoms	6 (14%)	62 (76%)	
Minor/mild	9 (21%)	10 (12%)	
Moderate to severe	27 (64%)	10 (12%)	
Q 4. Passing gas			<0.001
No Symptoms	6 (14%)	37 (45%)	
Minor/mild	13 (31%)	27 (33%)	
Moderate to severe	23 (55%)	18 (22%)	
Q 5. Constipation			<0.001
No Symptoms	8 (19%)	57 (70%)	
Minor/mild	18 (43%)	12 (15%)	
Moderate to severe	16 (38%)	13 (16%)	
Q 6. Diarrhea			<0.001
No Symptoms	23 (55%)	64 (78%)	
Minor/mild	6 (14%)	14 (17%)	
Moderate to severe	13 (31%)	4 (4.9%)	
Q 7. Loose bowel movements			<0.001
No Symptoms	17 (40%)	63 (77%)	
Minor/mild	9 (21%)	15 (18%)	
Moderate to severe	16 (38%)	4 (4.9%)	
Q 8. Hard stools			<0.001
No symptoms	10 (24%)	55 (67%)	
Minor/mild	15 (36%)	16 (20%)	
Moderate to severe	17 (40%)	11 (13%)	
Q 9. Urgent need to have bowel movement			<0.001
No Symptoms	12 (29%)	61 (74%)	
Minor/mild	11 (26%)	13 (16%)	

Moderate to severe	19 (45%)	8 (9.8%)	
Q 10. Feeling of incomplete emptied bowel			<0.001
No Symptoms	8 (19%)	57 (70%)	
Minor/Mild	16 (38%)	19 (23%)	
Moderate to severe	18 (43%)	6 (7.3%)	
Q 11. Fullness after starting meal			<0.001
No Symptoms	18 (43%)	65 (79%)	
Minor/Mild	13 (31%)	6 (7.3%)	
Moderate to severe	11 (26%)	11 (13%)	

Table 8. GI symptoms in Sjögren´s patients (n=42) compared to sex- and age-matched healthy controls (n=82). Analysis based on univariate logistic regression analysis

Characteristic	OR	95% CI	p-value
Q1. Abdominal pain			**<0.001**
No symptoms			
Minor/mild	2.61	0.96-7.44	
Moderate to severe	16.2	5.64-52.0	
Q2. Abdominal pain relieved by bowel action			**<0.001**
No symptoms			
Minor/mild	2.11	0.77-5.71	
Moderate to severe	17.8	6.04-62.2	
Q 3. Bloating			**<0.001**
No symptoms			
Minor/mild	9.30	2.79-33.7	
Moderate to severe	27.9	9.81-91.9	
Q 4. Passing gas			**<0.001**
No symptoms			
Minor/mild	2.97	1.03-9.38	
Moderate to severe	7.88	2.87-24.5	
Q. 5 Constipation			**<0.001**
No symptoms			
Minor/mild	10.7	3.92-31.8	
Moderate to severe	8.77	3.20-26.0	
Q. 6 Diarrhea			**<0.001**
No symptoms			
Minor/mild	1.19	0.38-3.37	
Moderate to severe	9.04	2.88-34.7	
Q 7. Loose bowel movements			**<0.001**
No symptoms			
Minor/mild	2.22	0.81-5.93	
Moderate to severe	14.8	4.75-57.2	

Q 8. Hard stools			**<0.001**
No symptoms			
Minor/mild	5.16	1.98 -14.1	
Moderate to severe	8.50	3.17-24.4	
Q 9. Urgent need to have bowel movement			**<0.001**
No symptoms			
Minor/mild	4.30	1.56-12.1	
Moderate to severe	12.1	4.47-35.7	
Q 10. Feeling of incomplete emptied bowel			**<0.001**
No Symptoms			
Minor/Mild	6.00	2.28-17.0	
Moderate to severe	21.4	6.95-75.8	
Q 11. Fullness after starting meals			**<0.001**
No symptoms			
Minor/mild	7.82	2.71-25.1	
Moderate to severe	3.61	1.35-9.82	

5. CONCLUSIONS

Deficiencies of fat-soluble vitamins are frequent in patients with chronic pancreatitis, but there is considerable heterogeneity between different studies. The highest rate for deficiency was noted when analyzing vitamin D. Only a few studies compared CP patients to a control group and based on these studies no increased risk for vitamin D deficiency was found in CP patients.

A high rate of osteopenia and osteoporosis was found in patients with chronic pancreatitis. Moreover, high prevalence rates of vitamin D deficiency and pancreatic exocrine insufficiency (PEI) were noted that were not correlated with osteopathy.
Female sex, age and BMI were associated with of osteoporosis in the whole cohort, while in male patients, the only risk factor associated with osteoporosis was vitamin K deficiency.

All patients with Crohn's disease with clinical remission that were included in our study had normal fecal elastase-1 levels indicating absence of PEI in this group of patients.

Based on our study, the great majority of patients with primary Sjögren´s syndrome suffered from IBS-like GI symptoms that cannot be attributed to pancreatic exocrine insufficiency.

6. GENERAL DISCUSSION

6.1 Paper I

By a systematic meta-analysis we determined the prevalence of deficiencies of fat-soluble vitamins in patients with chronic pancreatitis. Pooled prevalence of fat-soluble vitamins were shown to be high for vitamin D, E and E (57.6% and 29.2%, 16.8%, respectively). Pancreatic exocrine insufficiency with resulting maldigestion and malabsorption of fat-soluble vitamins is a well-defined complication in patients with advanced chronic pancreatitis. (90) In spite of this, previous studies published prevalence rates of vitamin deficiencies with large variations. To better assess the risk for vitamin deficiencies in CP patients, we performed a meta-analysis that strictly followed the recommendations defined by PRISMA.

The strength of the study was that all 12 studies were tested for publication bias via the Begg and Mazumdar test and for study quality by applying the Newcastle Ottawa Scale. Moreover, heterogeneity of included studies was tested. Testing for heterogeneity is crucial when conducting a meta-analysis as it is more difficult to draw conclusions if the included studies have a very heterogeneous study design. Noteworthy, the included studies of this meta-analysis exhibited significant heterogeneity. For example, cut-off values of vitamins varied in the studies.
Three of four fat-soluble vitamins were evaluated in this meta-analysis (vitamin D, E, A). Only one single study published results of vitamin K in CP patients and was not included in the analysis. Vitamin K is rarely measured in laboratories and INR-levels are serving as a substitute parameter which is increased in vitamin K deficiency.

Two different types of studies were included in this meta-analysis, namely cross-sectional studies/cohort studies and case-control studies. Surprisingly, only few case-control studies were available for further analysis although they are clearly preferable in order to determine if vitamin deficiencies can be attributed to chronic pancreatitis. To answer this question, a control group is advantageous. This can be exemplified by the results of vitamin deficiencies. In total, 57.6% (95% CI, 43.9-70.4%) of CP patients had vitamin D deficiency which is a clearly defined risk factor for osteopathy. Surprisingly, no statistical difference was found when comparing significance rates of CP patients with controls. In a recent systematic review and meta-analysis on the same topic no increased risk of vitamin D was observed in CP patients. (91)

Different factors can contribute to the fact that no significant difference was observed between CP patients and controls:
CP patients with early stage of chronic pancreatitis do not have developed PEI and consequently will not have an increased risk to develop vitamin D deficiency due to maldigestion. Not all included studies have clearly indicated the number of patients with proven pancreatic exocrine insufficiency.

Patients with advanced CP and established pancreatic exocrine insufficiency who receive a pancreatic enzyme replacement therapy (PERP) will usually no longer have maldigestion with impaired absorption of fat-soluble vitamins. This patient group with a proper oral treatment will have a similar risk for vitamin deficiency compared to controls. This could even be an explanation for the observation that no significant difference was noted when comparing vitamin D deficiency in CP patients with PEI versus CP patients without PEI. Interestingly, the prevalence of PEI varied significantly from 35 to 100% and in parallel, the percentage of patients receiving pancreatic enzyme supplementation varied from 46 to 100% on the basis of the included studies, demonstrating significant heterogeneity.
Meta-regression is another statistical method to address the problem of heterogeneous studies in meta-analysis which was not applied in our study. (92)

To get a better notion of the association of vitamin deficiency and PEI, only CP patients with newly diagnosed untreated PEI could be included in a future study. The disadvantage is that it would be much more difficult to include a large number of CP patients when applying this inclusion criterion. It might be even noteworthy, that the prevalence of vitamin D deficiencies are related to sun exposure and geographical areas. Consequently, deficiency rates differ significantly within Europe. (91)

The majority of studies (7/12) were conducted in Europe. It would be interesting to study in future studies if there are differences in vitamin deficiencies when comparing data from different continents. It has been taken into account that vitamin D deficiency is high in the general population and related to sun exposure. According to a study from France, vitamin D deficiency was more prevalent in the northern parts of France (93). Vitamin D deficiency is regarded as a worldwide problem with high prevalence rates in the population (94).

A limitation of the included studies is the limited information regarding ongoing alcohol-consumption in CP patients. Ongoing alcohol misuse is a well-established risk factor for

malnutrition and vitamin deficiencies (83). Consequently, the limited number of CP patients did not enable an analysis of vitamin deficiencies in relation to different etiologies which would clearly be of interest.

Although studies were tested for selection bias, we have to take into consideration that most studies were conducted at university hospitals suggesting the presence of a selection bias. One might hypothesize that CP patients at tertiary referral centers have more advanced CP with complications. On the other hand, CP patients at tertiary referral centers may have a better treated PEI-related maldigestion due to better follow-up and integration in multidisciplinary teams with dietitians.

When performing the meta-analysis only one study analyzed vitamin K levels in CP patients. (84) This motivated us to initiate a multicenter study in order to investigate vitamin K in this patient group. (95)

In summary, interesting questions are still unresolved and could be addressed in future studies:

1. How many patients with newly diagnosed untreated PEI have vitamin deficiencies compared to controls?
2. How many CP patients with PEI and ongoing enzyme replacement therapy have vitamin deficiencies? Are rates of deficiencies related to the daily oral dose of enzymes?
3. Are deficiency rates in CP patients higher in pateints with more advanced CP?
4. Is the risk vitamin deficiencies in CP patients dependent on the etiology of CP?
5. What is the risk for vitamin deficiencies in CP patients with ongoing alcohol misuse compared to abstinent CP patients with former alcoholism.

6.2 Paper II

In **Paper II** we conducted a large prospective multicenter study including 211 well-characterized patients with chronic pancreatitis in order to define the role of osteopathy and its risk factors in chronic pancreatitis.

There is evidence that osteoporosis has a high prevalence in CP with a pooled rate of 23% according to a meta-analysis from 2014. (82) The same meta-analysis demonstrated heterogeneity of study results with large variations of the prevalence rates of osteoporosis. Contributing factors for these variations were small patient numbers in previous studies and incomplete data regarding prevalence rates of exocrine pancreatic insufficiency and oral enzyme replacement therapy (PERT).

The pathophysiology of advanced chronic pancreatitis resulting in PEI with subsequent maldigestion and malabsorption of fat-soluble vitamins are well-characterized but the specific risk factors for osteopathy in chronic pancreatitis are ill-defined.

Osteopathy results from bone remodelling with increased resorption and resulting low bone mineral density and demineralisation. Several factors can contribute to osteoporosis: vitamin D and calcium malabsorption, therapy with glucocorticoids, smoking, physical inactivity, hormonal imbalances with low estrogen and chronic inflammation including IBD and cholestatic liver diseases. (96)

In the present study, chronic pancreatitis was classified according to the M-ANNHEIM classification which systematically assesses the etiology and severity of chronic pancreatitis (14) The classification is well-established and was applied in numerous clinical studies. Furthermore, the application of this classification is recommended by a recent EU guideline on the diagnosis and therapy of chronic pancreatitis. (17)

Bone density was prospectively examined by dual-energy X-ray absorptiometry which represents the gold standard for the diagnosis of osteopenia and osteoporosis.

Similar to previous studies a high percentage of patients demonstrated vitamin D deficiency (56%) and almost one-third patients (32%) had a vitamin K deficiency.

The strength of the study was that vitamin K was included in the analysis. There is accumulating evidence that the fat-soluble vitamin K is involved in bone homeostasis besides its contributory role in the production of coagulation factors by posttranslational modification. Vitamin K diminishes activity of osteoclasts by gamma-carboxylation of key proteins such as

RANK-L and NF-*k*B (11). It is of importance that vitamin K deficiency was observed in patients with pathological fractures, patients with Crohn's disease with osteopathy and cystic fibrosis patients. (97-99)

Vitamin K1 is the main isoform which is termed phylloquinone. The main source of vitamin K are green vegetables and fruits. In contrast, vitamin K2 (menaquinone) is produced by intracolonic bacterias (microbiome). (100, 101)

When analyzing the cohort of male CP patients by univariate and multivariate analysis vitamin K deficiency was the only risk factor associated with low bone mineral density with a high odds ratio of 5.28 (95 CI, 1.31-21-40, p=0.01). This association was not confirmed when performing a regression analysis on the basis of the whole cohort of 211 male and female CP patients. Here female sex was a significant risk factor for osteoporosis (OR 2.78, CI 95% 1.16-6.68, p=0.021). All female patients with vitamin K deficiency where shown to be postmenopausal - which is known to be correlated with osteoporosis. (102)

A thorough analysis of all available parameters did not demonstrate an association of vitamin D deficiency, PEI, fecal elastase, advanced disease or active smoking with osteoporosis.

The high rate of vitamin K deficiency in male patients with chronic pancreatitis is an important finding and we draw the conclusion that further and even larger prospective studies are required to better define the risk factors for osteoporosis in CP patients. Supplementation of vitamin K might be considered when treating patients with vitamin D deficiency.

Importantly, almost two-thirds of CP patients had osteopathy - with a rate of osteopenia of 42% and a rate of osteoporosis of 22%. This finding confirms the importance of the recommendation of international guidelines that CP patients represent a high-risk group for osteoporosis in need for dual-energy X-ray absorptiometry.(17) Vitamin deficiencies should be corrected and the therapy of osteoporosis should follow current guidelines to prevent the risk for fractures. (103, 104)

6.3 Paper III

The frequency of exocrine pancreatic insufficiency was studied in 20 patients with Crohn's disease. Pancreatic involvement in inflammatory bowel disease (IBD) is regarded as an extraintestinal manifestation (EIM). (105) EIM are frequent in IBD with an occurrence of 6% to 47% in IBD patients. The pathophysiology of EIM is still ill-defined but data suggest that mucosal inflammation triggers autoimmune responses due to cross reactivity with epitopes of intestinal bacteria. Several studies suggest that genetic risk factors contribute to EIM. The most important EIM include musculoskeletal forms (affecting joints), cutaneous forms (erythema nodosum, pyoderma gangrenosum) and oral lesions (aphthous stomatitis), ocular manifestations (scleritis, episcleritis, and uveitis) and hepatobiliary forms (PSC). (106, 107)

Pancreatic involvement in IBD has a large spectrum that involves simple elevation of pancreatic enzymes, acute and chronic pancreatitis, including autoimmune pancreatitis.

Population-based studies indicate that the risk for acute pancreatitis is higher in IBD patients. (108-110)

The increased risk in Crohn's disease can be explained by an increased risk for biliary pancreatitis due gallstones. A significantly decreased reabsorption of biliary salt in the terminal ileum is present in patients with ileocecal resection or active inflammation of the terminal ileum.

Anti-inflammatory medications (particularly azathioprine) trigger transient asymptomatic elevations of pancreatic enzymes (amylase/lipase) but can additionally induce acute pancreatitis with abdominal pain and/or morphological changes that are compatible with acute pancreatitis. Up to 10% of all patients with chronic pancreatitis have an autoimmune pancreatitis, which can be divided into IgG4 positive AIP type 1 and IgG4 negative AIP type 2. Particularly patients with type 2 AIP have a significantly increased risk to develop ulcerative colitis. Only limited data is available regarding the risk for chronic pancreatitis in IBD. Compared to the general population, alcohol plays a less prominent role in IBD-associated chronic pancreatitis.

Several studies evaluated the prevalence of exocrine pancreatic insufficiency with large variations (ranging from 18 to 80%). Results are summarized in **Table 9**.

Different tests were applied to investigate PEI.

Direct function tests (DFTs) were used to sample duodenal fluid after pancreas stimulation with hormones (secretin and/or pancreozymin) or a meal (Lundh test). Collecting duodenal fluid is performed by fluoroscopic placement of a catheter into the duodenum or by collecting duodenal fluid via aspiration with a gastroscope. DFTs enable the measurement of concentrations of bicarbonate and pancreatic enzymes but have the disadvantage of invasiveness.

This is one of the reasons that indirect function tests (IDFTs) such as the N-benzoyl-L-tyrosyl-p-aminobenzoic (NBT-PABA) test or the fecal-elastase test are preferred. The fecal elastase test is the recommended method to test for PEI according to a recent EU guideline and represents nowadays the most widely used method to evaluate the presence of PEI. (17) The test is an enzyme-based immunosorbent assay (ELISA), is non-invasive and only a very small sample of feces is required for analysis.

Based on our analysis in 20 patients with Crohn's disease, we could not confirm an increased risk for PEI in CD. A limitation of the study was the limited number of patients. In addition, all CD patients were in remission. According to previous studies, PEI seems to be related to extension of Crohn’s disease, to location (ileal inflammation) and to the presence of active inflammation. (52) Active jejunal inflammation may impair stimulation of the pancreatic secretion via decreased enterokinase activity. (111)

False-positivity of the fecal-elastase test has been taken into account: in watery diarrhea the test becomes positive due to dilution. (112) This is of particular importance when investigating PEI in IBD patients. In a study by Maconi, fourteen IBD patients with low FE-1 were followed up with a second FE-1 test after 4-6 months and normalization of FE1 was noted in two-thirds (10/14) of patients. (113)

We are well-aware of the limitations of the FE-1 test. Normal FE-1 levels can exclude the presence of PEI with high certainty but the cut-off values to define PEI are still subject to debate. In addition, the FE-1 test proved to have a low sensitivity to detect a mild to moderate exocrine pancreatic insufficiency. (114) Consequently, we cannot exclude asymptomatic impairment of pancreatic secretion in our patients with normal FE-1 tests.

Table 9. Studies investigating pancreatic dysfunction in IBD patients.

AUTHOR	YEAR	N	PATIENTS	TEST	RESULTS
Angelini(115)	1988	17	13 males, 4 females; mean age 34.6 years (range 17-70 years); mean duration of CD 36 months	Secretin-cerulein test	Bicarbonate and enzyme insufficiency in 4 out of 17 (23.5%) patients with CD and an enzyme insufficiency in 2 (11.7%) others
Hegnhoj(116)	1990	143	59 males, 84 females; mean age 37 years (range 15-80 years); mean duration of CD 76 months	Lundh meal test	Significantly decreased activities of amylase and lipase in duodenal aspirates. Disease activity and localization or extent of CD seems to be responsible for impaired pancreatic function
Heikius(117)	1996	46	Mean age of the whole group of IBD patients was 43.5 years and the male to female ratio was 0.94	Primary selection with NBT-PABA test and further evaluation with secretin test	12 patients with low NBT-PABA test; 3 patients with abnormal secretin test
Seibold(118)	1996	64	37 females and 27 males; mean duration of CD 8.8 years; mean age 38 years (range 16-68 years)	Chymotrypsin	Seven of the PAB-positive patients (27%) had impaired pancreatic function, in contrast to three of 38 PAB-negative patients (8%) ($p < 0.05$).
Barthet(119)	2005	71	In total 71 patients with CD were included; among them 49 patients without history of pancreatitis; mean duration of CD 10.3 years; median age 36 years	FE-1	FE-1 was low in 17% of patients without previous history of pancreatitis
Maconi(113)	2007	100	55 females; 45 males mean age 41.4 years	FE-1	14 patients with low FE-1; normalization of FE1 in 10 out of 14 patients after 4-6 months; no significant association was found between PEI and other clinical variables.
Malluta(120)	2019	51	28 females, 23 males; mean age 38 years (range 18-59 years); mean duration of CD 84 months	FE-1	4 (7.8%) patients with low levels of FE-1: all of them with normal EUS and MRI findings

6.4 Paper IV

According to our study with the inclusion of 57 patients, Sjögren´s syndrome does not appear to be associated with an increased risk for PEI. All patients who performed FE-1 testing and/or a ^{13}C-mixed triglycerides (^{13}C-MTG) breath test had normal results.

Several previous studies stated an increased prevalence of pancreatic exocrine dysfunction. (33, 65). Different diagnostic methods were applied in rather small cohorts of SS patients and frequently control groups were lacking. It is of note that PEI had not been strictly defined. We defined PEI as a significant reduction of enzyme activity in the GI tract resulting in maldigestion according to a current EU guideline. (17) According to this concept, minor reductions of enzyme activity do not play a significant clinical role.

In contrast, in our study of 57 patients with Sjögren´s syndrome, the most widely used test for diagnosing PEI - namely the fecal elastase-1 test - was in all patients normal. Moreover, in all tested patients the ^{13}C-mixed triglycerides (^{13}C-MTG) breath test was normal. The test delineates maldigestion of ^{13}C-marked triglycerides in the presence of a prominent lipase deficiency and is a recommended method to investigate PEI according to a recent EU guideline. (17)

As a limitation, only one-third of the included SS patients agreed to conduct a breath-test. To increase the acceptance rate, a short version of the 13C-MTG breath test was used which was shown to have similar rates of sensitivity and specificity. (11)

The aim of our study was to detect clinically relevant pancreatic exocrine insufficiency due to a prominent reduction of enzyme activity. Our chosen methods are not suitable to detect minor pancreatic dysfunction.

Importantly, we systematically evaluated GI symptoms in all SS patients and compared the prevalence and severity of 11 items with a control group that was derived from more than 1000 randomly contacted subjects from Stockholm (PopCol study). The large majority of patients had IBS-like GI symptoms consisting of feeling of incomplete emptying after defecation, relief of abdominal pain after bowel movement, constipation and diarrhea.

This is the first study that systematically investigated a large range of IBS-like GI symptoms in a well-characterized cohort of primary SS patients compared to a strict sex and age matched control. According to our data, SS patients harbor a significant risk of developing severe IBS-like GI symptoms. Based on our findings, the high prevalence of GI symptoms in SS cannot be attributed to exocrine pancreatic insufficiency.

7. FUTURE PERSPECTIVES

Meta-analyses and systematic reviews are valuable methods to summarize previous studies within a topic of interest before starting a new study in a similar area of research. We published our study in 2016 and it would be of great interest to conduct a new meta-analysis on the basis of larger and more homogeneous studies to generate more reliable results.

In our second study we included the analysis of vitamin K which is an important factor in bone metabolism. There is still limited data regarding vitamin K deficiency in this group of patients with chronic pancreatitis. Therefore, it is worthwhile to plan new prospective studies including age and sex matched controls in order to determine the risk for deficiencies of fat-soluble vitamins and micronutrients. This would enable us to get a more objective picture of deficiency rates in patients with CP compared to control subjects.
Besides measuring markers of maldigestion, such prospective studies should include the analysis bone mineral density, sarcopenia and other important risk factors (such as cardiovascular complications).

The following concrete questions may be addressed in future studies:
How many patients with newly diagnosed untreated PEI have vitamin deficiencies compared to controls?
How many CP patients with PEI and ongoing enzyme replacement therapy have ongoing vitamin deficiencies?
Are rates of deficiencies related to the daily oral dose of enzymes?
Are deficiency rates in CP patients higher in more advanced CP?
Is the risk vitamin deficiencies in CP patients dependent on the etiology of CP?
What is the risk for vitamin deficiencies in CP patients with ongoing alcohol misuse compared to abstinent CP patients with former alcoholism?
The important impact is information of PERT and the dosage and quality of life questions which is missing in our studies. Because of shorter median survival time and increased risks of comorbidity and mortality risks it would be an interesting aspect to do a long cohort study in this patient group with regular controls of fat soluble vitamins and micronutrients which affects both quality of life and comorbidity risk.

There is accumulating evidence demonstrating a prominent role of the intracolonic microbiome in modulating a large range of gastrointestinal diseases. Studies investigating microbiota in patients with CP and IBD patients could be of interest even when focusing on exocrine pancreatic insufficiency and its complications. It would be a promising idea to investigate possible correlations between osteoporosis, vitamin K levels and the microbiome.

Based on our study in patients with Crohn´s disease who were all in remission a new study with the inclusion of patients with Crohn´s disease and active small bowel inflammation would be of interest. This study could further clarify the concept that mucosal inflammation in the small bowel leads to pancreatic dysfunction and possible pancreatic exocrine insufficiency due to impaired stimulation of the pancreas.

Finally, future studies are warranted to delineate the underlying etiology of gastrointestinal symptoms in SS patients which may include studies of enteral dysmotility and interventional studies targeting IBS. We support the concept that gastroenterologists are included into the multidisciplinary team when taking care of patients with Sjögren´s syndrome.

8. POPULÄRVETENSKAPLIG SAMMANFATTNING

Kronisk pankreatit är en progressiv inflammatorisk sjukdom av bukspottkörteln som kännetecknas av fibros och irreversibla morfologiska förändringar som så småningom kan leda till permanent förlust av såväl exokrin som endokrin funktion.

Etiologin till kronisk pankreatit är multifaktoriell. De vanligaste faktorerna är alkohol och rökning. Andra orsaker är ärftliga (mutationer i *PRSS1* eller *SPINK1*), anatomiska (pankreas divisum, obstruktion av pankreasgången, pankreaskirurgi), immunologiska (autoimmun pankreatit) och sällsynta metabola (hyperkalcemi, toxiner) faktorer och medfödda tillstånd såsom cystisk fibros (CF) som beror på mutationer i genen *CFTR*.

Exokrin pankreasinsufficiens (EPI) uppstår när 90 % av det pankreatiska parenkymet förloras. Kliniska symtom på EPI uppvisas vanligtvis inte förrän nivåerna av matsmältningsenzymer i pankreas faller under 10 % av den normala nivån och den resulterande maldigestionen av de intagna näringsämnen leder till näringsbrist.

Ett bra screeningtest för att bekräfta klinisk misstanke om exokrin pankreasinsufficiens är mätning av elastas i feces (f-elastas). F-elastas är stabilt under hela passagen genom tarmen i kontrast till lipas och amylas som degraderas. F-elastas analysen är enkel, snabb, icke-invasiv, påverkas inte av samtidigt intag av pankreasenzymer och kan genomföras med ett enkelt avföringsprov. Generellt anses f-elastas <200 μg/g avföring som patologisk och förenlig med EPI.

Ett indirekt test som används för att diagnostisera EPI är en magnetkameraundersökning (MRCP) efter stimulering av pankreas sekretion med sekretin. Denna metod visualiserar volym av pankreassaft i tolvfingertarmen, men ger ingen direkt information om enzymfunktionen. Bilden hänvisar till vätskeproduktion (ekvivalent med bikarbonatsekretionen) som dock korrelerar väl med enzymproduktion.

^{13}C-MTG (mixed triglycerid) utandningstest anses vara ett mer känsligt test för att diagnostisera EPI än FE-1-mätning och som specifikt kvantifierar förmågan att metabolisera triglycerider. Vid EPI har utsöndringen av pankreassaft sjunkit under den nivå som krävs för en normal matsmältning. Vid ^{13}C-MTG utandningstest intas triglycerider som innehåller fettsyror som markerats med kol-isotopen ^{13}C (normalt kol är ^{12}C). Kol-isotopen ^{13}C är en stabil kol-isotop som förekommer naturligt, men i relativt låg koncentration. När de ^{13}C-

märkta triglyceriderna når tunntarmen bryts de ner av pankreasenzymet lipas till monoglycerider och fria fettsyror. Fettsyrorna tas upp av tarmslemhinnan och efter metabolism i levern bildas $^{13}CO_2$ som sedan andas ut och samlas i påsar för analys. Vid bristande lipasproduktion blir koncentrationen av $^{13}CO_2$ lägre i utandningsluften och exokrin pankreasinsufficiens kan på detta sätt påvisas och kvantifieras.

När diagnosen EPI är ställd får patienten en oral substitution av pankreasenzymer i form av kapslar som kompenserar enzymbristen.

I delarbete I genomförde vi en systematisk granskning och metaanalys av förekomsten av brist på **fettlösliga vitaminer hos patienter med CP**. Projektet utformades som ett internationellt samarbete mellan gastroenterologer från olika länder, däribland Estland, Tyskland, Italien, Polen, Spanien och Sverige. Databasen MEDLINE söktes fram till januari 2016 för case series och case-control studier som rapporterade förekomsten av eventuell fettlöslig vitaminbrist hos CP-patienter. Frekvensen av vitaminbristen analyserades och förekomsten av vitaminbrist mellan CP och kontroller jämfördes genom statistiska beräkningar (relativ OR och 95% konfidensintervall).
Tolv artiklar med totalt 548 CP-patienter inkluderades i denna metaanalys. De sammanlagda frekvenserna av vitamin A, D och E-brist var 16,8%, 57,6% och 29,2%. Endast en studie utvärderade vitamin K-brist. Pooled OR för vitamin D brist var jämfört med kontroller har varit 1.17 (95% CI 0.79 - 1.78).
Brist på fettlösliga vitaminer är frekvent hos CP-patienter, men det finns stor heterogenitet mellan olika studier. När det gäller vitamin D finns det bara få studier som hade en kontrollgrupp och denna metaanalys visade ingen uppenbar ökad risk hos CP patienter att ha vitamin D brist. Viktig är att nämna att information har varit ofta begränsad hur många av CP patienterna har haft en exokrin pankreasinsufficiens och hur många fick en oral behandling med pankreasenzymer. Större och mer homogena studier är nödvändiga för att bättre uppskatta förekomsten av fettlösliga vitamin brister, med särskilt fokus på vitamin K.

Målet i delarbete II har varit att utvärdera förekomsten av **osteoporos och osteopeni hos patienter med kronisk pankreatit** i en stor, homogen population enligt standardiserade diagnoskriterier. Det andra syftet med denna studie är att bedöma sambandet mellan bentäthet och kännetecken på kronisk pankreatit, och näringsmässiga parametrar, speciellt vitamin D och K. Projektet utformades som ett internationellt samarbete mellan gastroenterologer från

olika länder, däribland Estland, Tyskland, Italien, Polen, Spanien och Sverige. Det är en del av utbildnings- och forskningsprogrammet "Pancreas 2000". Vi genomförde en multicenter tvärsnittsstudie (P-BONE studie) och analyserade patienter med kronisk pankreatit där vi registrerade klinisk information och olika biokemiska variabler. Exokrin pankreasinsufficiens fastställdes genom fekal elastas-1 (FE-1). En systematisk bentäthetsmätning utfördes genom en DXA-undersökning (dual-energy X-ray absorptiometry/DEXA). Statistiska metoder genomfördes med hjälp av Fishers exakta test och genom t-test.
211 patienter med kronisk pankreatit inkluderades (67% män, medelålder 60 år). Osteopeni diagnostisterades i 42% av fallen och osteoporos i 22% av alla CP patienter.
43% av CP patienter har haft en bakomliggande alkoholöverkonsumtion, 18% av patienterna hade svår kronisk pankreatit och 56% hade exokrin pankreasinsufficiens. Medelvärdet av vitamin D var 21 ng/ml och 56% av fallen hade D-vitaminbrist. Det fanns ingen korrelation mellan D-vitaminnivåer, eller fekal elastas-1-nivåer och bentäthet (t-poäng på ryggrad eller lårben). Föreliggande data bekräftar en hög förekomst av osteopati hos patienter med kronisk pankreatit och ökad förekomst av osteoporos hos manliga patienter med K-vitaminbrist.

Crohns sjukdom (CD) är en kronisk inflammatorisk tarmsjukdom som främst drabbar tarmen men kan även drabba organsystem. Pankreas engagemang i CD kan orsakas av förändringar i pankreasgången, minskade hormonsignaler på grund av en inflammerad slemhinna i tarmen och av autoimmuna extraintestinala processer. Tidigare studier som undersökte prevalensen av EPI hos patienter med CD har visat motstridiga resultat och prevalensen varierar från 18 till 80 % i litteraturen. Kraftig varierande resultat kan bara delvis förklaras med att olika metoder har använts för att ställa diagnosen EPI och att olika urval av patienter har genomförts (selektionsbias). För att närmare kartlägga om det finns en risk för EPI hos CD patienter har vi genomfört en pilotstudie.
Vi genomförde en tvärsnittsstudie hos patienter med CD där vi registrerade klinisk information och biokemiska variabler. Alla inkluderade patienter har varit i remission. EPI fastställdes genom FE-1 som definieras som FE-1 <200 μg/g och 20 CD-patienter inkluderades (65 % män, medelålder 48 år). I alla patienter har varit FE-1 normal. I vår studie kunde vi visa att CD-patienter som är i remission inte verkar ha en ökad risk för exokrin pankreasinsufficiens. Vi bestämde oss för att inte fortsätta med studien då alla patienter hade normal FE-1. Större prospektiva studier är nödvändiga för att bättre uppskatta prevalensen av EPI som omfattar även patienter med aktiv tarminflammation.

Sjögrens syndrom (SS) är en kronisk autoimmun systemsjukdom med okänd etiologi som företrädesvis drabbar kroppens exokrina körtlar, såsom saliv- och tårkörtlar. Upp till 30% av patienter har även extraglandulära symptom. Patienter med primär SS har inga andra reumatiska sjukdomar, däremot kan SS vara kombinerad med tex. reumatoid artrit eller systemisk lupus erythematosus (sekundär SS). Pankreas och spottkörtlar är funktionellt och histologiskt jämförbara och därför ville vi undersöka om patienter med Sjögrens syndrom har en även en ökad risk för en nedsatt pankreasfunktion.
Vi genomförde en tvärsnittsundersökning hos patienter med primär SS som har rekryterats från en väl karakteriserad kohort i Stockholm. EPI fastställdes genom FE-1 och ^{13}C-MTG (mixed triglyceride) utandningstest. EPI definieras som FE-1 <200 μg/g och kumulativa värden ^{13}C-MTG (mixed triglyceride) utandningstest <20.9% efter 5 timmar.
Gastrointestinala symptom har vi utvärderat genom en etablerad frågeformulär (GSRS). Resultaten av frågeformuläret jämfördes med kön och åldersmatchade kontroller. Femtiosju patienter med primär SS inkluderades i studien som omfattade 92% kvinnor med en medianålder på 63 år. Totalt 87% (50/57) av SS-patienter testades för FE-1 och alla hade normala resultat. Alla patienter som genomgick en ^{13}C-MTG-BT (37%, 21/57) hade en normal kumulativ ^{13}C-utandning (> 20,9% efter 5 timmar).
Majoriteten av SS patienter har haft svåra gastrointestinal symptom och jämfört med kontrollgrupper fanns en signifikant skillnad i alla elva gastrointestinala parametrarna ($p<0,01$). Samma antal patienter noterade måttliga till svåra lösa avföringar (38%) och förstoppning (38%). Alla GI-symptom parametrar jämfördes mellan SS patienter och kontroller och de symptom med de största skillnader har varit: uppblåsthet (OR: 27,9; 95% CI: 9,81-91,9), känsla av ofullständig tömd tarm efter avföring (OR 21,4, CI 95 %: 6,95-75,8) och buksmärtor som minskade efter tarmtömningen (OD 17,8, 95% CI: 6,04-62,2).

I vår studie kunde vi visa att den stora majoriteten av SS-patienter hade IBS-liknande GI-symtom som inte kan hänföras till EPI.

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